HAEMOPHILIC
DISEASES
IN DENMARK

Translated from the Danish
by
Elisabeth Aagesen

HAEMOPHILIC DISEASES IN DENMARK

A Classification of the Clotting Defects in 78 Haemophilic Families

By

KNUD ERIK SJØLIN

M D

From the Institute of Human Genetics University of Copenhagen and the Biological Institute of the Carlsberg Foundation Copenhagen Denmark



BLACKWELL
SCIENTIFIC PUBLICATIONS
OXFORD

To Frederik and Soren

PREFACE

The investigations here presented were carried out while I was employed as voluntary assistant at the Biological Department of the Carlsberg Foundation 1955–1958

Within the past 20 years or so several members of the staff of the Department have been engaged in studies on the clotting of blood and the fibrinolytic system of the organism The present work is to be regarded as part of this comprehensive and important research scheme the inspiring leader of which is Tage Astrup Ph D It was in fact Dr Astrup who prompted me to this work by introducing me to the intricate problems of the coagulation process I wish to thank Dr Astrup most sincerely for this and for the free and independent working conditions offered me as well as for his positive and instructive criticism during my daily work

A large proportion of the patients investigated were traced through the Medico-Genetic Registry of the University Institute for Human Genetics My thanks are due to Professor Tage Kemp M D Director of the Institute for his permission to make use of the Registry and other facilities and I also thank Professor Kemp for much valuable advice during the preparation of my work

Much of the material examined was collected while I worked as assistant physician in the Department of Pathology The Finsen Institute and Radium Station I am grateful to Chief Physician Johannes Clemmesen M D Head of the Department partly for his organisation of my daily work so as to make it possible for me to carry through the investigation and partly for his never failing interest and encouragement

To Dr Mogens Hauge and Mr Arne Nielsen biostatistician both of the University Institute for Human Genetics I am obliged for instructive discussions on genetic and statistical problems

Mrs Erna Laursen nee Zoffman yielded invaluable assistance in most of the laboratory investigations

Especially I wish to thank my numerous colleagues all over the country who took the trouble to answer my inquiries or in other ways placed their knowledge at my disposal Without their disinterested contributions it would have been impossible for me to carry through this investigation

Further I extend my sincere thanks to the Danish haemophiliacs for their readiness to co operate I hope that they will be equally co operative in future investigations which very often will not lead to results of direct practical value to the patients concerned

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Knud Erik Sjølin

CONTENTS

Chapter VII

Chapter VIII

Chapter IX

Chapter X

Chapter XI

Chapter XII

Chapter XIII

Chapter XIV

Chapter XV

Chapter VII

Chapter VIII

Chapter IX

Chapter X

Chapter XI

Chapter XII

Chapter XIII

Chapter XIV

Chapter XV

Chapter VII

Chapter VIII

Chapter IX

Chapter X

INTRODUCTION

Pavlovsky s finding in 1947 that blood from one haemophiliac could correct the clotting defect in blood from another haemophiliac aroused a great interest in a mapping of these defects in haemophilia Several reports have been published since on different defects of the coagulation system of haemophiliacs *Rosenthal* (1954) *Soulier & Larrieu* (1954) *Beaumont Caen & Bernard* (1954) *Verstraete* (1955) and *Biggs & Macfarlane* (1957) mention the relative frequencies of such defects but no comprehensive investigations have been reported so far into the incidence of and heredity in the individual clotting defect

The works on haemophilia published in Scandinavia are mainly concerned with the symptomatology and inheritance of the disease (*Søren Hansen* 1886 *Andreassen* 1943 *Skold* 1944) After the syndrome of haemophilia had been demonstrated to be caused by various defects of the coagulation system it was found appropriate to re-examine the Danish haemophiliacs with a view to studying the qualitative clotting defect as well as the symptomatology of the individual forms and the inheritance of these

Object of the Investigation

The object of the present investigation was 1) to collect information as far as possible on all haemophiliacs in Denmark 2) to attempt to determine the qualitative clotting defect in these patients 3) to study the symptomatology and prognosis of the different forms of haemophilia and 4) to clarify the modes of inheritance of the individual types of the disease

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Chapter I

THE PROCESS OF NORMAL COAGULATION

The Classical Theory of Blood Clotting

Our knowledge concerning the clotting of blood has been greatly extended within the past 10–20 years Year by year we have become increasingly familiar with the apparently differing factors included in the process of coagulation Within the past ten years or so the number recognized has risen from four to about twelve The fact that the same factor goes by different names in the various haematological schools (see the table fig 1) adds further to the diffi culty of following the development within the field of humoral biology and pathology

A brief review will be given below of the development that has led to our present knowledge about the blood clotting Particular importance is attached to recent works on the earliest phases of blood clotting Moreover reference may be made to surveys by *Wohlisch* (1929 1940) *Astrup* (1944 1950) *Biggs & Macfarlane* (1957) and *Macfarlane* (1957)

For clearness sake it is expedient to divide this development in a number of periods (*Astrup* 1944 1950)

First Period

The first period started in 1859 with *Denis* (quoted by *Astrup* 1944) detec tion of fibrinogen and ended in 1905 when *Morawitz* advanced his theory about the blood clotting a theory which still holds good in principle

According to Morawitz classical theory the blood clotting takes place in two phases

First phase Prothrombin + thrombokinase + Ca++ → thrombin

Second phase Thrombin + fibrinogen → fibrin

Prothrombin present in blood plasma is converted into thrombin (*Alexan der Schmidt* 1872) by an activating substance thrombokinase which is liberated from damaged tissue or blood cells This conversion requires the presence of Ca++ (*Arthus & Pagès* 1890)

The clotting enzyme thrombin converts the fibrinogen contained in plasma into fibrin independently of the presence of Ca++ (*Hammarsten* 1896–1897)

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Nomenclature used in the present work	Synonyms	Deficiency disease
Christmas factor *(Biggs & al)*	– Factor IX *(Koller)* – Plasma thromboplastin component (PTC) *(Aggeler & al)* – Antihaemophilic globulin B *(von Cramer Matter & Loeliger)* – Platelet co-factor II *(Seegers)* – Plasma thromboplastic factor B (PTF B) *(Aggeler & al)*	Christmas disease Haemophilia B PTF B deficiency Deuterohaemophilia PTC deficiency Haemophilia like state A
Factor X	– Factor X *(Koller)*	
Hageman factor	– Factor XII	Hageman trait Hageman s disease
Plasma thromboplastin antecedent (PTA) *(Rosenthal)*	– Plasma thromboplastic factor C (PTF-C) *(Aggeler)*	PTA deficiency Rosenthals syndrom PTF-C deficiency Tritohaemophilia Haemophilia C Haemophilia like state B

Second Period

50 years elapsed before Morawitz theory obtained universal recognition The first disagreements were due to differences of opinion with regard to the nature of the tissue factor This factor was as stated called thrombokinase by some writers by others cytozyme and by most American writers thromboplastin Some investigators found this activating substance in aqueous extracts of the tissues where it was heat labile Others found it in alcoholic extracts where it was heat stable In 1912 this discrepancy was explained by the fact that thrombokinase was found to consist of an active lipoid bound on protein (*Bordet Howell Zak* (quoted by *Wohlisch* 1929)) This has since been confirmed by *Chargaff Bendich & Cohen* (1944)

Recognition of Morawitz theory was however further delayed because American workers especially adhered to two other theories regarding the clotting of blood advanced by *Mills & Guest* (1921) and *Howell* (1935)

The former writers who revived *Wooldridge s* theories (1883) were of the opinion that a tissue factor which they named tissue fibrinogen reacted with calcium and fibrinogen to form fibrin However this theory has never been proved experimentally

Howell took anticoagulants to play a very important part in the coagulation process prothrombin in the circulating blood being bound on an anticoagulant (heparin) He believed the prothrombin to be liberated by the tissue thrombokinase binding the anticoagulant and the prothrombin then to be converted into

Fig 1

Nomenclature table

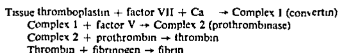
Nomenclature used in the present work	Synonym	Deficiency disease
Fibrinogen *(Denis)*	– Factor I *(Koller)*	Hypofibrinogenaemia Afibrinogenaemia
Prothrombin *(Alexander Schmidt)*	– Factor II *(Koller)* – Thrombogen *(Morawitz)* – Thrombozym *(Nolf)* – Serozym *(Bordet)* – Prothrombin B *(Quick)*	Hypoprothrombinaemia
Thromboplastin *(Howell)*	– Factor III *(Koller)* – Thrombokinase *(Morawitz)* – Cytozym *(Bordet)* – Thrombokinin *(Lenggenhager)*	
Calcium	– Factor IV *(Koller)*	
Proaccelerin *(Astrup Owren)*	– Factor V *(Owren)* – Labile factor *(Quick)* – Prothrombin A *(Quick)* – Thrombogen *(Nolf)* – Plasma accelerator globulin *(Ware & Seegers)* – Prothrombin accelerator *(Fantl & Nance)* – Plasma prothrombin conversion factor *(Stefanini)*	Hypoproaccelerinaemia Owren s disease Parahaemophilia
Accelerin *(Owren)*	– Factor VI *(Owren)* – Serum accelerator globulin *(Ware & Seegers)* – Serum accelerator *(Stefanini)*	
Proconvertin *(Owren)*	– Factor VII *(Koller)* – Stable factor *(Stefanini)* – Co-thromboplastin *(Mann)* – Serum prothrombin conversion accelerator (SPCA) *(de Vries & Goldstein)* – Prothrombin conversion accelerator (PCA) *(Owen & Bollman)*	Hypoproconvertinaemia Alexander s disease
Antihaemophilic factor (AHF) *(Brinkhous)*	– Factor VIII *(Koller)* – Antihaemophilic globulin (AHG) *(Patek & Taylor)* – Antihaemophilic globulin A *(von Cramer Matter & Loeliger)* – Platelet co-factor I *(Seegers)* – Plasma thromboplastic factor A (PTF A) *(Aggeler)* – Thromboplastinogen *(Quick)* – Thrombocytolysin *(Brinkhous)* – Facteur antihémophilic A *(Soulier)* – Thrombokatalysin *(Lenggenhager)*	Classical haemophilia Haemophilia Haemophilic A PTF A deaficiency

suggesting existence of yet another coagulation factor influencing the thrombin generation This factor was provisionally named co-factor V (*Owren* 1947) but was later called proconvertin (*Owren* 1951) factor VII (*Koller Loeliger & Duckert* 1951) co-thromboplastin (*Mann* 1949) serum prothrombin conversion accelerator (S P C A) (*de Vries Alexander & Goldstein* 1949) stable factor (*Stefanini* 1953) In Owren s opinion proconvertin does not act as an accelerator in the prothrombin formation but is necessary for the quantitative conversion of prothrombin *Koller et al* (1951) on the other hand showed that factor VII (proconvertin) acts as an accelerator in this process

The result of *Jacox* (1949) work suggests that factor VII unlike factor V acts as co-factor in the conversion of brain tissue into active thromboplastin the thromboplastic activity of brain tissue being increased after incubation with serum containing factor VII but not factor V

Returning to Morawitz scheme from 1905 this will have to be modified though disagreement prevails as to how the components of the prothrombin group (prothrombin proaccelerin (factor V) and proconvertin (factor VII)) react with each other (see fig 2) Tissue thromboplastin (tissue thrombokinase) combines with proconvertin (factor VII) in the presence of Ca^{++} to form a complex (convertin complex 1) which again combines with accelerin (activated proaccelerin factor V) to form yet another complex (complex 2 prothrombinase) which activates prothrombin into thrombin Thrombin then converts fibrinogen into fibrin *(Owren)*

Fig 2



Within recent years yet another factor has been demonstrated This is the Stuart Prower factor (*Graham & Hougie* 1956 *Telfer Denfer & Wright* 1956) which is necessary for tissue thromboplastin to be able to convert prothrombin into thrombin and also required for the formation of plasma thromboplastin (*Bachmann Duckert Greiger Baer & Koller* 1957)

Fifth Period

The investigations of the tissue thromboplastin had long diverted attention from the fact that normal blood withdrawn carefully without admixture of tissue juice clots quickly

Blood undergoing spontaneous physiological clotting is able to produce a thromboplastin which becomes activated when touching a foreign surface The thromboplastic activity of the blood is most often named plasma thromboplastin (*Biggs & Macfarlane* 1953) which is not a definite substance but a function of several plasma factors (*Biggs Douglas & Macfarlane* 1953)

thrombin by Ca^{++} alone This process has never been observed however (*Nolf* 1945 *Owren* 1947 *Milstone* 1948)

Diverging theories about the blood clotting were also advanced by some European writers within this period (e g by *Nolf* 1908 *Stuber & Lang* 1930)

All in all we may say that this second period of coagulation research re presented a transitory stagnation with regard to developing a proper under standing of the coagulation process because of the various diverging theories and hypotheses

Third Period

In the middle of the thirties a series of papers were published in America which rejected Mill s theories and returned to Morawitz classical theory (*Smith Warner & Brinkhous* 1934 *Quick* 1935 a b *Eagle* 1935) The latter had been maintained throughout by European writers (*Bordet Mellanby and Wohlisch*) Likewise the fundamental importance of Howell s anticoagulants was rejected

In 1935 *Henrik Dam* discovered the vitamin K and *Schønheyder* noticed its influence on the clotting of chicken blood

In 1937 *Patek & Taylor* found in normal plasma the antihaemophilic globulin capable of shortening the clotting time of haemophilic blood both *in vitro* and *in vivo*

Fourth Period

The fourth period comprises the mapping of the factors of the prothrombin group

The period started in 1943 when *Quick* demonstrated that the prothrombin time in stored oxalated plasma increased with the time of storage and that addition of small amounts of fresh plasma from a dicoumarol treated animal normalized the prothrombin time Quick concluded from this observation that fresh plasma contains in addition to prothrombin a labile factor which is destroyed by storage In 1944 *Owren* described a haemorrhagic diathesis caused by deficiency of a previously unknown coagulation factor factor V (*Owren* 1947) later called proaccelerin (*Astrup* 1950 *Owren* 1951) This factor is identical with Quick s labile factor

The same factor was found independently of Owren by *Ware Guest & Seegers* (1947) who named it Ac globulin (accelerator globulin) and by *Fantl & Nance* (1946) who called it prothrombin accelerator

According to *Owren* (1947) factor V (proaccelerin) is necessary for the conversion of prothrombin into thrombin The rate of this reaction as well as the produced amount of thrombin depends largely on the existing amount of proaccelerin *Astrup* as early as 1941 while investigating the significance of the autocatalysis in the blood clotting (*Owren* 1947) felt convinced of the existence of a factor influencing the rate of thrombin generation

In 1946 and 1947 *Owren* found variations in the rate of thrombin formation

Spaet Aggeler & Kinsell in 1954 mentioned a possible fourth plasma thromboplastin component a statement which however they subsequently withdrew (1957)

Finally *Ratnoff & Margolius jr* and *Ratnoff & Colopy* in 1955 reported on a factor in the thromboplastic system of plasma which was given the name of Hageman factor The Hageman factor was found accidentally as neither the first patient after whom the factor was named nor the great majority of the other cases published so far presented signs of haemorrhagic diathesis despite the pronounced clotting defect

The intermediary reactions between the AHF Factor V Christmas factor platelets and calcium preceding the thromboplastin formation have been studied particularly by *Biggs Douglas & Macfarlane* 1953 a b) *Owren* (1954) *Quick* (1954) *Bergsagel & Biggs* (1955) *Hougie* (1955 a) *Koller* (1955 b) *Bergsagel & Hougie* (1956) *Douglas* (1956 a b) and *Seegers & Johnson* (1956) The investigations of the Oxford school gave the result that the Christmas factor is activated at an early stage by contact with a foreign surface thereafter to react with the AHF and platelets This product combines with factor V to form thromboplastin As stated above the PTA as well as the Hageman factor and possibly Koller s factor X also contribute towards the thromboplastin formation *The thromboplastin generation in plasma is possibly started* by admixture of small amounts of tissue thromboplastin (*Astrup* personal communication)

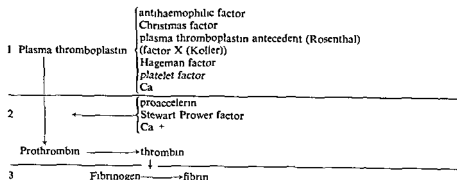
To tabulate the process of blood clotting on the basis of our present knowledge we may still start from Morawitz theory though in a somewhat modified form (see fig 3)

The thrombokinase in Morawitz scheme is to be replaced by the blood s own thrombokinase (plasma thromboplastin)

Fig 3

Morawitz extended coagulation scheme

Note that the scheme does not indicate the mutual reaction of the coagulation factors



As early as 1912 *Collingwood & MacMahon* suggested that plasma itself contains thromboplastin in inactive form They supposed that the precursor of the thromboplastin which they called prothrombokinase came from the platelets at the beginning of the clotting They also found this process to be accelerated by serum which they took to contain preformed thromboplastin or an activator of the prothrombokinase

The thromboplastic activity of the platelets differs however appreciably from that of tissue thromboplastin Addition of normal platelets to haemophilic plasma will rarely reduce the clotting time of this whereas *addition of tissue* thromboplastin even from haemophiliacs will normalise the clotting time

Quick (1947) and *Brinkhous* (1947) found independently that the platelets could only promote clotting in the presence of the antihaemophilic globulin *Quick* regarded AHG as a precursor of thromboplastin (thromboplastinogen) which was activated by the platelets while *Brinkhous* believed that thromboplastin was liberated from the platelets after these had been dissolved by AHF The latter acted as thrombocytolysin *Conley Hartmann & Morse (1949)* using silicone treated glass tubes demonstrated that platelet free plasma contains a soluble precursor of thromboplastin, which is activated by contact with glass surfaces Further researches into the plasma thromboplastin were however conditional on the investigations of the clotting defect in haemophilia

Pavlovsky showed in 1947 that the clotting time for blood from a haemophiliac had been reduced after transfusion of 100 ml of plasma from another haemophiliac with a greatly prolonged clotting time This observation suggested *qualitative differences of the clotting defect between different haemophiliacs*

Five years elapsed however before the first subgroup of hamophiliacs was distinguished Almost simultaneously in 1952 *Aggeler White Glendening Page Leake & Bates* and *Biggs Macfarlane Dacie Pitney & Merskey* reported on an additional factor essential to the normal clotting This factor was named plasma thromboplastin component (PTC) in the U S A and Christmas factor in Great Britain Deficiency of this factor was *found to effect a haemorrhagic diathesis clinically identical with haemophilia*

Shortly after the detection of the Christmas factor American workers became aware of a new factor likewise essential to the normal clotting (*Rosenthal Dreskin & Rosenthal* 1953) This factor was given the name of plasma thromboplastin antecedent (PTA) PTA deficiency is believed to be the cause of a mild haemophilia like clinical picture seen in both female and male members of some families (*Rosenthal Dreskin & Rosenthal 1953 Frick 1954 Rosenthal Dreskin & Rosenthal* 1955 *Ramot Angelopoulus & Singer* 1955 a)

In 1954 a previously unknown coagulation factor was demonstrated in serum This was designated as factor X (*Duckert Fluckiger Matter & Koller* 1955) It resembles the Christmas factor in some respects but differs from this in others For instance it can be prepared of plasma from a patient with Christmas disease and it is *less stable than the Christmas factor*

Chapter II

THE CLOTTING DEFECT IN HAEMOPHILIA

Haemophilia belongs to the plasmatically induced haemorrhagic diatheses These diseases are characterized haematologically by 1) prolonged clotting time 2) normal prothrombin time 3) delayed conversion of prothrombin into thrombin

The slow clotting was first demonstrated by *Liston* (1839) *Wright* (1893) noticed that the clotting time was prolonged *Addis* (1911) that the prothrombin concentration was normal and *Addis* (1911) as well as *Brinkhous* (1939) that the conversion of prothrombin into thrombin was greatly delayed

Fibrinogen

It is universally agreed that the clotting defect in haemophilia is not referable to the last phase of the coagulation process i e the conversion by thrombin of fibrinogen into fibrin The fibrinogen in haemophilic blood is normal quantitatively as well as qualitatively (*Sahli* 1905 *Weil* 1905 *Addis* 1911 *Lewis Davidson Minot Soulier Tagnon & Taylor* 1946)

Prothrombin

Sahli (1905) believed the prothrombin content in haemophilic blood to be normal This was confirmed by *Addis* (1911) who however found a greatly delayed conversion of prothrombin into thrombin He ascribed this to a qualitative change of the prothrombin The theory of a qualitative defect of the prothrombin was rejected by *Howell* (1914) in whose opinion there is a deficiency of prothrombin which he supposed to originate in the thrombocytes However *Howell & Cekada* (1926) found the prothrombin in haemophilic blood to be normal both qualitatively and quantitatively This has since been confirmed by several writers (*Quick Stanley Brown & Bancroft* 1935 *Quick* 1935 a *Lewis Davidson Minot Soulier Tagnon & Taylor 1946* among others)

The plasma thromboplastin requires presence of proaccelerin and the Ste wart Prower factor to be able to convert prothrombin into thrombin It is doubtful whether proconvertin is necessary to this process (*Ackroyd* 1956 *Seegers & Johnson* 1956) According to the modified Morawitz scheme the clotting is thus divided in three phases as shown in fig 3

This scheme has the advantage of affording a basis for a direct classification of the plasmatically determined haemorrhagic diatheses in three groups (*Deutch* 1955, *Stefanini & Dameshek* 1955 *Astrup & Sjølin* 1957)

1) One group comprising disorders of the formation of plasma thrombo plastin the haemophilic diseases 2) one group with defects of the prothrombin complex and 3) one group presenting disturbances of the conversion of fibrino gen into fibrin The diagnosing of these diseases will be reviewed in chapter III

As for other theories regarding coagulation reference may be made to *Nolf* (1908) *Bordet* (1912 1920) *Howell* (1918 1935) *Stuber & Lang* (1930) *Owren* (1947) and *Biggs & Macfarlane* (1953)

divided with regard to their quality Several writers have stated that haemophilic platelets possess an increased resistance *Morawitz & Lossen* (1908) were of the opinion that the platelets and the leukocytes give off their thrombokinase too slowly and in too small amounts *Howell* (1914) held that the clotting defect ought to be referred to a qualitative change of the platelets these having a diminished prothrombin content

According to *Fonio* (1914 1915) the slower clotting rate in haemophilic blood than in normal is due to the thrombocytes giving off their thrombozyme (thrombokinase) more slowly than normal platelets In Fonio s experiments the clotting promoting actions of haemophilic and normal platelets differed only in haemophilic plasma but not when the two platelet suspensions were added to normal plasma On the other hand according to *Fonio* extracts of haemophilic platelets seemed to be inferior to extracts of normal platelets with regard to promoting clotting

As late as 1932 *Fonio* declared that the reduced coagulability of haemophilic blood was due to the failing ability of the platelets to prompt liberation of thrombokinase so that the first phase of the coagulation process with formation of thrombin proceeded too slowly *Fonio* showed in some interesting experiments that the clotting time for a platelet poor haemophilic plasma could be normalized by adding a suspension of normal platelets washed in physiological saline A suspension of haemophilic platelets did not have the same effect The clotting times were 10 and 90 minutes respectively

Minot & Lee (1916) demonstrated that normal platelets in suspension reduced the clotting time for haemophilic blood The same was found for haemophilic platelets though to a much smaller extent even with very large amounts These writers found on the other hand that platelet suspensions from haemophilic blood and from normal blood had the same accelerating influence on the clotting of normal plasma

Minot & Lee concluded that haemophilic platelets are qualitatively defective giving off prothrombin at a slower rate than normal platelets The authors erroneous conclusion concerning the platelet function was due in the first instance to the fact that they believed to determine the prothrombin time by recalcifying oxalated plasma

Klinger (1918) was of the opinion that the coagulation defect in haemophilia was not referable to the platelets as these accelerated the clotting of normal blood to the same extent as the normal platelets Further the clot retraction which is a platelet function was normal in haemophilic blood

According to *Feissly & Fried* (1924) the ability of normal platelets to correct the coagulation defect in haemophilia is accountable for by adsorption of normal plasma on the surface of the platelets Washing of the platelets reduced their coagulation promoting ability

Howell & Cekada (1926) demonstrated an increased resistance of haemophilic platelets during the clotting

Calcium

Wright (1893) claimed that haemophilia is associated with calcium deficiency because he found a reduced clotting time for haemophilic blood to which had been added calcium chloride This hypothesis was however, rejected by *Morawitz & Lossen* (1908) *Addis* (1911) *Howell* (1913 1914) *Howell & Cekada* (1926) and *Christie Davies & Stewart* (1926–1927)

Thromboplastin

In 1905 *Sahli* on the basis of determinations of the clotting time in haemophilic blood withdrawn in different phases of the disease as well as on the basis of theoretical reflections advanced the hypothesis that the defective haemostasis in haemophilia is due to the vascular walls giving off insufficient thrombokinase for the conversion of prothrombin into thrombin He explained the shortening of the clotting time for haemophilic blood after addition of normal blood in the way that the latter brought a supply of thrombokinase liberated from the cells of the added blood *Sahli* concluded from this that the vascular walls as well as the blood cells of haemophiliacs are unable to give off thrombokinase or possibly contain this substance in smaller quantities

Addis (1911) and *Schloessmann* (1912) thought that there was no difference between the blood of haemophilics and that of normals with regard to the amounts of thrombokinase produced from tissue and of formed elements *Howell* (1914) denied the existence of thrombokinase In his view thrombokinase is a substance or substances *long known under the name of zymoplastic or* thromboplastic substances consisting of a phosphatide This was supposed to accelerate the coagulation process by neutralizing antithrombin but not by any direct action on prothrombin *Howell* (1914) and *Minot & Lee* (1916) had moreover remarked that the clotting time for haemophilic blood was reduced when the vein was entered uncleanly owing to admixture of tissue thromboplastin Howell obtained a similar effect by adding cephalin to the blood In agreement with this a decreased thromboplastic activity has never been demonstrable in tissue from haemophiliacs (*Gressot* 1912 *Minot & Lee* 1916 *Lowenburg & Ruberstone* 1918) (See however p 168)

Brown (1952) found that an extract of brain tissue from haemophiliacs was an equally effective coagulant in haemophilic blood as normal brain tissue *Quick* (1954) claimed that tissue from haemophiliacs contains the same amount of thromboplastin as normal tissue

Platelets

Thrombocytes are present in a normal number in haemophilic blood (*Sahli* 1905 *Howell* 1914 *Minot & Lee* 1916 *Wohlisch* 1923) Opinion has been

as normal *Quick* (1949) saw no change in the coagulability of haemophilic blood after addition of crushed platelets

Mond & Singer (1951) succeeded in normalising the clotting time for haemophilic blood by adding a homogenate of crushed platelets The prothrombin consumption also improved though not always significantly

Sjølin (1957 a) investigating horse plasma lacking the Hageman factor found the thrombin generation to improve by adding the plasma s own platelets after these had been washed in physiological saline

Holburn Carroll & Tocantins (1957) examined blood samples from a group of patients with characteristic haemophilia and found a decreased platelet activity to be the predominant abnormality The clotting time and the thromboplastin formation became normal after addition of normal platelets to the patients plasma but not after addition of the same amount of platelets from patients Infusion of a platelet rich plasma into these patients resulted in a normal clotting time and thromboplastin formation The improvement lasted for three days

In the literature available conflicting views have thus been advanced concerning the function of the platelets in haemophilia This may be due to the various writers having examined haemophiliacs who presented typical clinical pictures and had a prolonged clotting time but who had clotting defects differing in quality Besides high platelet concentrations obtained by adding platelet suspension or freeze dried platelet derivatives will prolong the recalcification time in plasma Dilution to normal concentrations will render the recalcification time normal again (*Klein Farber Freeman & Fiorentino* 1956)

Remaining Components of the Prothrombin Complex

As stated previously it is universally agreed that the prothrombin content of haemophilic blood is normal Determination of the prothrombin content by Quick s method usually gives normal values (*Quick* 1935 a) indicating that the remaining components of the prothrombin complex factor V (proaccelerin) and factor VII (proconvertin) are contained in normal quantities *Owren* (1947) also established the fact that the factor V content in haemophilic blood is normal *Frank Bilhan & Ekren* (1950) found that plasma from a patient with factor V deficiency was able to correct the clotting defect in haemophilic plasma

Plasma Thromboplastin

Sahli demonstrated as early as 1905 that haemophilic blood clotted like normal blood after addition of tissue extract This means, as stated that the prothrombin time for haemophilic blood is normal Consequently the phases of the coagulation process that have been started by thromboplastin will behave normally On the other hand the part of the coagulation which is to function

Christie Davies & Stewart (1926–1927) found the clotting time for haemophilic blood to decrease to a certain minimum by adding increasing amounts of water The same occurred after whipping of haemophilic blood These observations were explained by increased liberation of prothrombin from the decaying platelets

Fuchs & Falkenhausen (1930) adhered to the view of an abnormal stabilisation of the thrombocytes in haemophilia While normal thrombocytes tended to stick to foreign bodies (glass, bacteria carbon) thrombocytes from haemophilic blood did not agglutinate till after the clotting had started These workers also found that haemophilic platelets did not have the same clotting promoting effect on normal plasma as normal platelets However the more the haemophilic platelets were washed the greater became their clotting accelerating ability

Govaerts & Gratia (1931) found the clotting of haemophilic plasma to be accelerated by adding normal platelets Platelets from haemophilic blood accelerated the clotting of normal platelet poor oxalated plasma equally as normal platelets but the former were abnormally stable in their own plasma Destruction of platelets in platelet rich haemophilic plasma proved to shorten the clotting time This was done in three ways 1) Freezing of platelet rich plasma to −16° C After thawing the clotting time fell from 7 hours to 30 minutes 2) Addition of distilled water to platelet rich haemophilic plasma followed by addition of NaCl in substance until the sample was isotonic again gave a clotting time of 10–30 minutes whereas it was 3 hours in the plasma to which physiological saline had been added at once 3) By first rendering haemophilic plasma hypertonic with NaCl and next isotonic with distilled water a clotting time of 70 minutes was obtained against 200 minutes in control samples

Birch (1931 1932) demonstrated an increased osmotic resistance of haemophilic platelets Whereas normal blood platelets behaved like erythrocytes in the presence of hypotonic saline haemolysing at a concentration of about 0 4 % NaCl the haemophilic platelets did not change in hypotonic solutions In hypertonic solutions a concentration of 5 % NaCl was required for their disintegration If the haemophilic platelets were crushed in a mortar and then added again to the blood from which they had been removed the clotting time was greatly shortened in most cases to the normal

Andreassen (1943) was unable to corroborate Birch s results regarding the increased osmotic resistance of haemophilic thrombocytes but found them to change at NaCl concentrations between 0 34 and 0 40 % *Lenggenhager* (1936) attributed no specific function to the platelets in the clotting of blood even if the clotting of haemophilic blood became normal by adding a suspension of normal platelets because he obtained the same result by adding kaolin powder to haemophilic blood *Wright* (1946) found less agglutinability in haemophilic platelets than in normal

Quick & Stefanini (1948) found haemophilic platelets to be equally active

intravenous injection Similar results were achieved by *Patek & Taylor* (1937) who found the globulin substance to be absent or present in greatly diminished amounts in preparations of haemophilic blood They also showed that the globulin fraction was precipitated with the fibrinogen They produced their preparation by dialysing plasma with distilled water

Lozner & Taylor (1939) employed a similar procedure They managed to maintain a normal clotting time in a haemophiliac by injecting plasma globulin every six hours

These results have since been confirmed by *Minot Davidson Lewis Tagnon & Taylor* (1945) and *Lewis Tagnon Davidson Minot* & Taylor (1946) among others *Lewis Soulier & Taylor* (1946) found that a solution of Cohn s plasma fraction I to which the antihaemophilic activity is attached preserved its activity after the fibrinogen had been removed

The antihaemophilic globulin AHG (*Lewis Tagnon Davidson Minot & Taylor* 1946) (thromboplastinogen (*Quick* 1947) thrombocytolysin (*Brinkhous* 1947) the antihaemophilic factor (*Brinkhous & Graham* 1954) factor VIII (*Koller* 1954) plasma thromboplastic factor A PTF A (*Aggeler White & Spaet* 1954) plasma thromboplastic factor (*Stefanini* 1953) thrombokatalysin (*Lenggenhager* 1946)) is found in Cohn s fractions I and III tolerates heating to 56° C is soluble in water and is rapidly destroyed about 50 per cent within 12 hours at room temperature It is stable in the frozen state and after freeze drying It is precipitated at 25–33 per cent saturation with ammonium sulphate and is not adsorbed by inorganic adsorbents It is present in plasma but not in serum Bovine plasma contains about 16 times more antihaemophilic factor than human plasma (*Macfarlane Biggs & Bidwell* 1954)

The Christmas Factor (Plasma Thromboplastin Component)

As stated previously *Pavlovsky* found that blood from one haemophiliac was able to correct the clotting defect in blood from another haemophilic patient Five years elapsed however before an explanation could be given of this observation

Biggs Douglas Macfarlane Dacie Pitney Merskey & O Brien (1952) and *Aggeler White Glendening Page Leake & Bates* (1952) almost simultaneously reported seven and one case respectively of a haemorrhagic diathesis indistinguishable from haemophilia both clinically and on ordinary haematological examinations Investigations comprising determination of the clotting time prothrombin consumption test and thromboplastin generation test revealed however that the clotting defect in these patients unlike that in another group of haemophiliacs was not reduced by adding antihaemophilic factor adsorbed plasma or Seitz filtered plasma neither were blood samples from these patients able to normalise each other s clotting defect which on the other hand was compensated by adding blood from other haemophiliacs The clotting de

in the absence of tissue thromboplastin must be pathological in haemophilic blood As stated previously normal blood will clot spontaneously within a few *minutes even if withdrawn without admixture of tissue juice* The clotting of haemophilic blood may take hours to set in Normal blood seems to have the power of converting prothrombin into thrombin, whereas haemophilic blood lacks this power as seen by the slow conversion of prothrombin into thrombin (*Addis* 1911)

Weil (1905 quoted 1946) was *presumably the first to demonstrate that* intravenous injection of fresh serum into haemophiliacs gave a perfectly normal clotting time which persisted for several days (Christmas patient?) *Addis* (1911) found that addition of 20 per cent normal plasma to haemophilic blood *in vitro gave a normal clotting time Further, he observed that small amounts* of a globulin fraction prepared by acid precipitation of normal plasma accelerated the clotting of haemophilic blood The results of these experiments suggest that the globulin fraction of normal plasma contain a coagulation factor *which is absent in haemophilic plasma* Addis was *unfortunately of the* opinion that his globulin fraction consisted mainly of prothrombin He therefore concluded that a qualitative prothrombin defect exists in haemophilic blood although his globulin fraction was able to correct the clotting defect in such *small concentrations that a prothrombin defect could probably be excluded* Addis misinterpretation of the results of his otherwise excellent experiments delayed for 20 to 30 years our present view concerning the clotting defect in haemophilia

Feissly demonstrated *in 1924 that transfusion of cell and platelet free* plasma into haemophiliacs normalised the clotting time Feissly took the delayed clotting of haemophilic blood to be due to an abnormality of the thrombin forming plasma system

Govaerts & Gratia (1931) showed that *the clotting of haemophilic plasma* could be accelerated in three ways 1) by adding tissue extract 2) by adding normal platelets and 3) by adding normal plasma in small amounts (1/25 vol) They regarded the platelets in haemophilic plasma as abnormally stable and believed that their destruction *could be promoted by adding a plasma factor* other than prothrombin This factor was heat labile was present in phosphate adsorbed plasma and able to pass through a Berkefeld filter It was presumably identical with the antihaemophilic factor

Patek & Stetson (1936) *confirmed Feissly s* (1924) *and Govaerts & Gratia s* (1931) observations having found that addition of very small amounts (1/40–1/200 ml) of cell and platelet free plasma shortened the clotting time of haemophilic blood Berkefeld filtration of the plasma did not reduce its clotting *accelerating activity The active factor* antihaemophilic globulin demonstrated by *Addis* (1911) was found in the acid precipitated globulin fraction

Pohle & Taylor (1937) found that the globulin fraction prepared from normal plasma shortened the clotting time *of haemophilic blood after intramuscular or*

bulin or $BaSO_4$ adsorbed plasma which does not contain the *Christmas factor* The clotting defect was also corrected by adding blood from a patient with AHF deficiency as well as from one with Christmas factor deficiency

PTA (plasma thromboplastin antecedent) was found to be stable in plasma and serum for 2 years at $-10°$ and $-20°$ C Sterile plasma stored for 4 months at room temperature had a normal PTA activity Storage at $-15°$ C increased the activity of PTA in both normal plasma and in plasma with a moderate PTA deficiency Heating to 60° C caused partial destruction of the factor It was found in $BaSO_4$ adsorbed plasma and serum but could also be eluted from barium sulphate after the adsorption Seitz filtered plasma adsorbed only little PTA Plasma thromboplastin antecedent precipitates mainly at 25–33 per cent saturation with ammonium sulphate and is present in Cohn s fraction IV–1 The electrophoretic mobility of PTA is intermediate between that of gamma globulin and that of beta globulin (*R L Rosenthal* 1955) It is evident from what has been stated above that the PTA factor has properties reminiscent partly of the AHF (salting out electrophoresis adsorption *Seitz filtration*) and partly of the Christmas factor (Cohn s fraction IV electrophoresis stability adsorption) The possibility has therefore been ventilated that the patients with PTA deficiency may have a mild combined deficiency of AHF and Christmas factor too (*Verstraete & Vandenbroucke* 1955 *Verstraete* 1955 *Biggs & Macfarlane* 1957) This hypothesis was rejected by *Ramot Angelopoulus & Singer* (1955 a) The view of the latter writers regarding the properties of the PTA factor differs somewhat from that of Rosenthal (see the table fig 4)

Relatively few patients with PTA deficiency have been described so far

Fig 4

The properties of the plasma thromboplastin antecedent

	A to Ros h l	Acc. to R m t e l
Activity in normal plasma stored at −20 C	increased	unchanged
Influence of tmp on activity in normal plasma or serum	60 C for 20 min no activity	58 C for 10 min Activity considerably decreased
Activity after Seitz filtration	moderately decreased	considerably decreased
Activity in different fractions precipitated with (NH) SO	saturation 0–25 minimum 25–35 / maximum 33–50 small	saturation 0–20 / considerable 20–33 maximum 33–50 / small
Contents in Cohn s fraction	I and III small amounts IV maximum II IV_4 V nil	I nil

fect could also be corrected by adding serum from both normals and other haemophiliacs

The lacking factor the Christmas factor, as it was named by *Biggs et al* after their first patient or the plasma thromboplastin component (PTC) (*Aggeler et al*) is present in fraction IV of normal plasma (according to *Cohn*) and follows the beta globulins during electrophoresis It is adsorbed by Al $(OH)_3$ and $BaSO_4$ is precipitated by ammonium sulphate at 33–50 per cent saturation is stable at room temperature but is destroyed when heated to 56° C for 10 minutes It is found in plasma and serum from haemophiliacs with AHF deficiency and from normals

The haemophilia like disease Moena described by *Koller et al* (1950) is not identical with Christmas disease but is a mild AHF deficiency (*Koller* 1954)

Since 1952 numerous cases of Christmas disease have been reported (*Schulman & Smith* 1952 *Lewis & Ferguson* 1955 *Creveld & Paulssen* 1953 *Poole* 1953 *Frick* 1954 *Rosenthal* 1954 *Torregrosa Ortis & de Rivera* 1954 *Soulier & Larrieu* 1954 *Ramot Angelopoulus & Singer* 1955 *J Gormsen* 1956 *Sjølin* 1956)

Graham (1956) states that 69 cases of Christmas disease had been published up to 1956

Biggs et al (1952) found the disease to be inherited as a sex linked recessive disorder just like classical haemophilia This was borne out by *Lewis & Ferguson* (1953) *Frick* (1954) *M C Rosenthal* (1954) and *Koller* (1955 a)

Graham (1956) on the basis of the literature available found that among 247 haemophiliacs 207 had AHF deficiency and 40 i e 16 per cent Christmas factor deficiency

Sjølin & Videbæk (1956) and *Hardisty* (1957) have found a reduced content of the Christmas factor in blood from some women with a haemorrhagic diathesis

Rosenthal s Factor (Plasma Thromboplastin Antecedent)

Shortly after the first descriptions of Christmas disease Rosenthal Dreskin & Rosenthal (1953) reported to have found yet another factor necessary for the normal coagulation Absence or deficiency of this factor likewise gave rise to a haemophilia like clinical picture though as a rule with somewhat milder signs and symptoms A predominant sign was that of profuse haemorrhage following dental extraction *Rosenthal et al* s first patients were two sisters and their mother s brother

In these patients the clotting time was prolonged and the prothrombin conversion delayed The thrombocyte count prothrombin capillary resistance and bleeding time were normal *The clotting defect could be corrected by* adding either normal serum which does not contain the antihaemophilic glo-

authors named the disorder tertartohaemophilia and the new factor plasma thromboplastic factor D (PTF-D)

This new factor obtained only a short life Further investigations showed that the patient s plasma lacked the Christmas factor and contained an anti coagulant of low activity (*Spaet* 1957)

Circulating Anticoagulants

As the final cause of the clotting defect in haemophilia mention should be made of the circulating anticoagulants even though these probably play a secondary part and tend to mask the primary clotting defect (See above)

Circulating anticoagulants prevent formation of plasma thromboplastin but has no influence on the formed plasma thromboplastin thrombin or prothrombin (*Hougie* 1955 b)

Circulating anticoagulants may occur in three categories of patients 1) haemophiliacs 2) parturient women and 3) some patients falling outside 1) and 2) especially in association with immune reactions

Only group 1) will be dealt with here *Pohle & Taylor* (1938) were the first to demonstrate that injection of globulin into haemophiliacs gave rise to a refractory period after the usual initial fall of the clotting time

Munro & Jones (1943) followed a haemophilic patient through four years During this period the patient was given frequent transfusions at first with a favourable effect on the bleeding tendency but afterwards causing exacerba tion of this tendency At the same time the patient s plasma inhibited the clotting in normal plasma Similar reactions have since been demonstrated by *Craddock & Lawrence* (1947)

Frommeyer Epstein & Taylor (1950) investigated 22 patients with classical haemophilia and found five who were refractory to transfusions and to treat ment with plasma protein fractions The authors detected an anticoagulant and precipitins against different plasma fractions with AHF activity presumably developed by immunisation *Penalver Holburn Carroll & Tocantins* (1957) could demonstrate no antibodies refractory to transfusion in blood from haemophiliacs *Hougie* (1955 b) and *Verstraete* (1955) have found 17 cases reported in the literature of classical haemophilia with development of anticoagulant Such cases have been described since by *Speer Hill Maloney Roberts & Prager* (1956) among others

Soulier & Larrieu (1953) found anticoagulants in two patients with defi ciency of the Christmas factor *Bergna & Pavlovsky* (1956) state that inhibitors occur relatively more often in Christmas patients than in patients with AHF deficiency

The circulating anticoagulants are generally heat stable 65° C for 5 minutes (*Hougie* 1955 b) 70° C for 10 minutes (*Verstraete* 1955) stable at refrigera tor temperature (*Hougie* 1955) They tolerate long storing at −20° C (*Verstraete*

Ramot Angelopoulus & Singer (1955 a) have reported three cases two women and one man diagnosed by thromboplastin generation test and prothrombin consumption test *Oehme & Hagette* (1955) mention two brothers with the disease diagnosed by thromboplastin generation test, and *Scardigli & Guidi* (1956) one case diagnosed by clotting time determination and prothrombin consumption test The mother s brother of this patient had haemophilia with AHF deficiency *Biggs & Macfarlane* (1957) found three patients with PTA deficiency

R L Rosenthal (1954) and *Frick* (1954) noticed that PTA deficiency, unlike classical haemophilia and Christmas disease is transmitted to females, too

Hageman Factor

Ratnoff & Margolius jr (1955) have described an asymptomatic clotting defect localised in the thromboplastic system of the plasma The defect could be corrected by a factor contained in adsorbed serum and tolerating heating to 56° C for 30 minutes The factor could be precipitated from this serum at 25–40 per cent saturation with ammonium sulphate

In addition to the first three patients described one man and two women having this clotting defect, which was discovered accidentally five other cases of the disorder have been reported in the literature all asymptomatic and all except one occurring in males (*Frick & Hagen 1956 Ramot Singer Heller & Zimmermann* 1956 *Sjølin* 1957 b)

Larrieu Soulier & Culot (1957) have described a similar clotting defect in a woman who was liable to ecchymoses but displayed no other signs of haemorrhagic diathesis

Finally *Sjølin* (1957 d) has reported a case of haemorrhagic diathesis in which the clotting defect could be corrected by heated readsorbed serum This patient was therefore referred to the Hageman group

Very little has been written about the inheritance of the Hageman trait *Margolius & Ratnoff* (1956) believe that the disorder is due to an autosomal recessive gene The maternal grandfather of the patient described by *Sjølin* (1957 d) had been a bleeder The patient s mother displayed no haematological signs of a haemorrhagic diathesis

The Fourth Plasma Thromboplastin Component

Spaet Aggeler & Kinsell in 1954 described a haemorrhagic diathesis in a man aged 31 In his family there were found both male and female bleeders The clotting defect was claimed to be caused by deficiency of a plasma and serum factor contained in Cohn s fractions III and IV and precipitated by normal plasma at 50 per cent saturation with ammonium sulphate The factor was found to be heat labile stable on storage and not adsorbed by barium sulphate Blood transfusion did not improve the coagulability of the blood The

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1955) are not adsorbed and are not dialysable (*Hougie* 1955 b *Verstraete* 1955) Anticoagulants are present in the gamma globulin fraction (*Craddock* & *Lawrence* 1947), but can be extracted with ether from serum (*Verstraete* 1955)

Dilution of the plasma may cause the anticoagulant effect to disappear (*Soulier* & *Larrieu* 1953)

Speer Hill Maloney Roberts & *Prager* (1956) mention an inhibitor of a lipoid character which proved to differ somewhat in quality having disappeared after freezing of platelet rich plasma but not after freezing of platelet poor plasma

Tocantins Theory of the Clotting Defect in Haemophilia

Tocantins and his collaborators have advanced the view in a series of papers that classical haemophilia is not due to deficiency of the antihaemophilic factor but haemophilic blood as also normal blood contains a thromboplastin inhibitor (1942–1943 1943 1951) The antithromboplastic activity is believed to be five to eight times greater in haemophilic plasma than in normal plasma (*Tocantins* 1942–1943) The inhibitor when isolated acts as an antagonist to the cephalin half of the thromboplastin (*Tocantins* 1943) Tocantins view is supported by the fact that dilution with saline shortens the clotting times for normal whole blood and plasma as well as for haemophilic whole blood and plasma In haemophilic blood a normal clotting time can even be obtained by a suitable dilution (*Tocantins Carroll* & *Holburn* 1951) This result of dilution could not be expected if haemophilia were caused by deficiency of a thromboplastic factor

Tocantins view has in some measure been supported by other workers (*Nilsson* & *Wenckert* 1954 *Verstraete* 1955)

Graham & *Barrow* (1957) have tested Tocantins theories on blood from haemophilic dogs and explained the shortening of the clotting time after dilution of the plasma by changes in the ionic strength

Chapter III

DETECTION OF CLOTTING DEFECTS

Fibrinogen

Defects of the coagulation system (see the scheme for blood clotting fig 3 p 19) may be due to absence or deficiency of fibrinogen Fibrinogen can be detected qualitatively by adding 0 2 ml of bovine thrombin (100 units per ml) to 0 5 ml of citrated plasma If fibrinogen is present a clot will form instantaneously In afibrinogenaemia no clot will be formed and in hypofibrinogenaemia thin fibres will form but no proper clot

A quick estimate of the fibrinogen concentration can be obtained by using *Schneider*s method (1952) The principle of the method is that of adding thrombin solution to a number of whole blood or plasma dilutions and recording the fibrin precipitation in the highest dilution of the sample Comparison with the result of a corresponding assay of a normal plasma will give information on a possible fall of the fibrinogen concentration

Inhibitors

Inhibitors among which heparin is the most important may interfere with the conversion of fibrinogen into fibrin The simplest way of testing for inhibitors is to add a little of the plasma concerned to normal plasma and determine the clotting time after addition of thrombin or merely after recalcification If the clotting time is not prolonged significantly the sample contains no abnormal amounts of inhibitors

If an existing inhibitor interferes with the formation of thromboplastin we find decreased conversion of prothrombin into thrombin measured by prothrombin consumption test thrombin generation test or thromboplastin generation test for instance The clotting defect can be corrected by adding excess of thromboplastin Quick s prothrombin time is normal or very slightly prolonged

In case an inhibitor interferes with the thromboplastin formed Quick s prothrombin time is prolonged and can be only partially normalised by adding excess of tissue thromboplastin (*Stefanini & Dameshek* 1955)

Heparin acts partly as an antithrombin partly and especially as an inhibitor of the conversion of prothrombin into thrombin (*Howell* 1924–1925)

This inhibitory activity is counteracted by thromboplastin (*Astrup* 1944) basic proteins, such as protamine sulphate (*Fischer* 1935 *Chargaff & Olson* 1937–1938 *Jorpes Edman & Thaning* 1939) and toluidine blue (*Holmgren & Wilander* 1937)

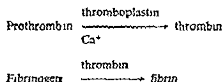
Hyperheparinaemia is often accompanied by a slightly prolonged prothrombin time (*Quick* 1955)

Defects of the coagulation system may, however also be caused by a lowered thrombin concentration or by a reduced formation of thrombin (see fig 5) This may be due to a lowered concentration of prothrombin or of thromboplastin

The Prothrombin System

Prothrombin is converted into thrombin by thromboplastin in the presence of calcium ions The thrombin thus formed converts fibrinogen into fibrin (see fig 5)

Fig 5



In the latter process the clotting time is approximately in inverse ratio to the thrombin concentration (*Astrup & Darling* 1942) *Quick* s introduction of *prothrombin time determination (1935) rendered possible demonstration of a* reduced prothrombin concentration in plasma The test consists in adding 0 1 ml of brain extract (thromboplastin) and 0 1 ml of $CaCl_2$ to 0 1 ml of plasma and then determining the clotting time The clotting time has thus been rendered independent of the thromboplastin concentration of the plasma sample and a prolonged prothrombin time indicates a reduced concentration of the factors of the prothrombin system (prothrombin proaccelerin proconvertin) Normalisation of Quick s prothrombin time after addition of small amounts of fresh adsorbed plasma suggests factor V deficiency in the plasma sample Normalisation of the prothrombin time after addition of stored serum lacking prothrombin and factor V suggests factor VII deficiency Prothrombin can be determined directly by using Russell viper venom instead of thromboplastin in the sample (*Hjort Rapaport & Owren* 1955)

Owren (1949) stabilised the coagulation system with regard to fibrinogen inhibitors and factor V by adding adsorbed bovine plasma *Thus the result of* prothrombin time determination according to Owren indicates the prothrombin proconvertin concentration

Quick s simple method of prothrombin determination is of very great value

If the prothrombin time is normal prothrombin proaccelerin and proconvertin are present in normal amounts The prothrombin time is normal in haemophilia thrombopenia and von Willebrand Jurgens disease

A prolonged prothrombin time is first of all found in association with deficiency of prothrombin proaccelerin and proconvertin Further it is seen in conditions of fibrinogen deficiency and hyperheparinaemia as well as Stuart Prower factor deficiency

Plasma Thromboplastin

Methods of investigating defects of the plasma thromboplastin have especially been developed since 1947 when *Quick* described his prothrombin consumption test However *Addis* as early as 1911 employed a thrombin generation test which likewise is suitable for investigations of these defects *Astrup & Fischer* (1936) studied the plasma thromboplastin formation in clotting chicken plasma

Plasma thromboplastin consists of 1) AHF (*Patek & Taylor* 1937) 2) Christmas factor (*Biggs Douglas Macfarlane Dacie Pitney Merskey & O Brien* 1952 *Aggeler White Glendening Page Leake & Bates* 1952) 3) plasma thromboplastin antecedent (*Rosenthal Dreskin & Rosenthal* 1953) 4) Hageman factor (*Ratnoff & Margolius* jr 1955 *Ratnoff & Colopy* 1955) 5) possibly factor X (*Koller* 1955 b) 6) a thrombocyte factor (*Brinkhous* 1947 *Quick* 1947 *Conley Hartmann & Morse* 1949 *Macfarlane & Biggs* 1953) and 7) calcium ions (*Morawitz* 1905 *Bergsagel & Hougie* 1956) see fig 3

Biggs & Douglas (1953) introduced thromboplastin generation test for direct measurement of the plasma thromboplastin formation For this test a washed platelet suspension is mixed with fresh adsorbed oxalated plasma (containing AHF and factor V) and serum (containing Christmas factor and factor VII) After recalcification of the mixture a specimen of this is transferred at one minute intervals to a sample of platelet free plasma or prothrombin fibrinogen mixture recalcified at the same time The clotting times obtained are compared with a standard curve based on dilution of plasma thromboplastin

Blood with AHF deficiency or PTA deficiency will give an abnormal thromboplastin formation by this method when the adsorbed plasma comes from the patient If the thromboplastin formation becomes abnormal when the serum used originates from a haemophilic patient this patient has Christmas disease or PTA deficiency

The thromboplastin generation test has been extensively used for diagnosing classical haemophilia (deficiency of antihaemophilic factor) (*Biggs & Douglas* 1953 *Verstraete* 1955 *Miale & Wilson* 1956) and Christmas disease (*Biggs & Douglas* 1953 *Verstraete* 1955 *Ramot Angelopoulus & Singer* 1955 *de Vries Kettenborg & Van der Pol* 1955 *Miale & Wilson* 1956) The test has in most cases given satisfactory results *Biggs & Macfarlane* (1957) state however that the method may fail in mild cases of these two diseases

The literature reports only few cases of deficiency of plasma thromboplastin antecedent (PTA) diagnosed by the thromboplastin generation test *Biggs & Macfarlane* (1957) investigated three patients with a haemorrhagic diathesis in whom the dominant inheritance the signs and symptoms as well as the laboratory findings resembled those in the patients with PTA deficiency de scribed by *Rosenthal Dreskin & Rosenthal* (1953) In one patient the result of the thromboplastin generation test suggested combined slight antihaemophilic factor and Christmas factor deficiency In the second patient the test gave varying results suggesting presence of now one defect and now the other The third patient apparently had slight Christmas factor deficiency

Oehme & Hagette (1955) diagnosed deficiency of plasma thromboplastin antecedent by the thromboplastin generation test in two brothers Using this method the thromboplastin generation became normal by adding either barium sulphate adsorbed normal plasma or normal serum *Miale & Wilson* (1956) likewise found the thromboplastin generation test to be useful for diagnosing PTA deficiency

In a few instances the thromboplastin generation test has been employed for diagnosing the Hageman defect (*Ratnoff & Margolius* jr 1955 *Ramot Singer Heller & Zimmermann* 1956) The thromboplastin generation became normal by replacing the patient s adsorbed plasma or serum by the corresponding control samples

The thromboplastin generation test has been used for diagnosing combined haemophilia i e concurrent deficiency of the AHF and the Christmas factor (*Verstraete & Vandenbroucke* 1955 *Verstraete* 1955)

In cases of functional platelet defects (thrombasthenia') the thrombo plastin generation test likewise shows a diminished thromboplastin generation (*Biggs & Douglas* 1953 *Holburn Carroll & Tocantins* 1957)

Finally the thromboplastin generation test reveals thromboplastic activity in the presence of inhibitors (*Biggs & Douglas* 1953 *Miale & Wilson* 1956)

From a theoretical aspect the objection may be raised that it seems in appropriate for diagnostic purposes to work with a mixture containing such an unphysiological component as serum the properties of which differ from those of plasma and of whose composition we actually know relatively little Likewise considering the uncertainty prevailing as regards the function of the platelets during the clotting of haemophilic blood it may seem inappropriate to introduce a normal washed platelet suspension in the coagulation mixture This may mask a clotting defect (see p 101)

Duckert Fluckiger Isenschmied Matter Vogel Meng & Koller (1954) observed in fact that the serum factors have a very pronounced accelerating effect on the thromboplastin generation *Bergsagel & Biggs* (1955) found the activity of the Christmas factor to be less in plasma than in serum

Miale & Wilson (1956) in a very thorough investigation studied the in fluence of various facts on the result of the thromboplastin generation test They

demonstrated among other things that by substituting saline for plasma in the incubation mixture a considerably greater thromboplastin activity was obtained than in experiments where serum was replaced by saline The same workers also found thrombin formation to occur already in the incubation mixture so that the test actually gives no isolated impression of the thromboplastin formation

Determination of Clotting Time

Measurement of the clotting time for blood gives an expression of the ability of the blood to change spontaneously *in vitro* from the liquid to the clotted state The test is quite unspecific as any disorder of clotting whether due to deficiency of a clotting factor or to presence of an inhibitor will prolong the clotting time In the former situation the clotting time becomes normal after addition of small amounts of normal blood whereas in the latter it remains prolonged

Clotting time determinations are only comparable if the extravascular factors acting on the coagulation process are kept constant These are 1) the temperature 2) the technique of blood sampling 3) any contact surfaces of the blood

The temperature should be kept at a constant level as the speed of clotting increases parallel with the temperature up to 37° C (*Bürker* 1907 1913 *Andreassen* 1943 *Biggs & Macfarlane* 1957)

At the blood sampling great care must be taken to avoid admixture of tissue juice The cannula must enter the vein cleanly Admixture of tissue juice to haemophilic blood may shorten the clotting time from several hours to a few minutes If the blood is sucked out with a record syringe froth formation should be avoided

The extravascular surfaces of contact, such as glass tubes and cannulae ought as far as possible to be constant An enlarged contact surface may partly promote the activation of clotting factors (e g Christmas factor and platelets) and partly accelerate the adsorption of the formed thrombin Any unnecessary shaking of the tubes should be avoided The calibration of tubes and cannulae ought to be constant In cases of hypercoagulability or minor clotting defects it may be expedient to use silicone treated tubes and cannulae (*Brinkhous* 1947 *Conley Hartmann & Morse* 1949)

A special form of clotting time determination is that of measuring the calcium clotting time or the recalcification time for diluted plasma The procedure described by *Howell* is that of diluting plasma with an equal part of saline and then adding the optimum amount of calcium chloride Dilution of the plasma will however shorten the clotting time (*Andreassen* 1945) compared with that for untreated blood Clotting time determination on haemophilic blood by this method will likewise give lower values This is in agreement with the observation that dilution of haemophilic blood effects shortening of

its clotting time (*Howell* 1914 *Christie Davies & Stewart* 1926–1927 *Tocantins Carroll & Holburn* 1951 *Feissly* 1934 *Graham* 1957)

Clotting time determination has no doubt been the most frequently employed analytical method in haemophilia since *Wright* (1893) demonstrated a prolonged clotting time in this disease

The clotting time being most often prolonged in haemophilia this finding together with the tendency to spontaneous bleeding and the sex linked recessive inheritance previously established the diagnosis (*Schloessmann* 1930 *Howell* 1939 *Quick* 1941 *Merskey* 1950)

Within recent years however a normal clotting time has not infrequently been observed in haemophilic blood (*Merskey* 1950 *Merskey* 1951 *Schmid* 1951 *Quick* 1952 *Graham McLendon & Brinkhous* 1953) *Merskey* (1951) found a normal clotting time in ten out of 51 haemophiliacs In eleven the clotting time was just above the upper normal limit The same author in 1951 found normal clotting times in haemophiliacs belonging to eight different families

In consequence of these observations opinion has been divided regarding the relationship between the length of the clotting time and the clinical manifestation of haemophilia

Weil (1906) adhered to the view of a parallelism between the prolongation of the clotting time and the bleeding tendency This view was shared by *Addis* (1911) *Schloessmann* (1912 1930) and *Merskey* (1950) *Sahli* (1905) and *Morawitz* (1930) on the other hand found the bleeding tendency to be fairly independent of the clotting time This view was shared by *Hecht* (1941–1942) *Schiller Neligan & Budtz Olsen* (1948) *Andreassen* (1943) using *Burker*s method (1907 1913) for clotting time determination found by studying 17 haemophiliacs that the length of the clotting time afforded no bases for conclusions regarding the intensity or prognosis of the disease

The failing correlation between clinical signs and clotting defect is seen particularly in cases with the Hageman trait (*Ratnoff & Margolius* jr 1955 *Ratnoff & Colopy* 1955 *Frick & Hagen* 1956 *Ramot Singer Heller & Zimmermann* 1956 *Sjølin* 1957 b) where even very long clotting times are often attended by no clinical signs whatever

Clotting Time Determinations and Classification of the Different Forms of Haemophilia

The first division of the haemophilic syndrome was as stated based on clotting time determination (*Pavlovsky* 1947)

Determination of the clotting time has since been a very important test for classification of haemophiliacs usually combined with prothrombin consumption test thromboplastin generation test and less frequently thrombin generation test

No detailed account will be given here of the works dealing with this problem but reference may be made to papers by *Aggeler White Glendening Page Leake & Bates* (1952) *Biggs Douglas Macfarlane Dacie Pitney Mer skey & O Brien* (1952) *Rosenthal Dreskin & Rosenthal* (1953) *Rosenthal* (1954) and *Soulier & Larrieu* (1954) among others

Summarizing we may say about the clotting time determination that it is a very simple but unspecific method of estimating the coagulability of the blood and that in connection with other tests it is a valuable aid to classification of a clotting defect Demonstration of a normal clotting time does not preclude the presence of a clotting defect

Prothrombin Consumption Test

When normal blood clots 85 per cent of the prothrombin is immediately converted into thrombin (*Brinkhous* 1939 *Quick* 1947 *Brinkhous* 1947 *Quick* 1949) However in 1939 *Brinkhous* as previously *Addis* showed that in haemophilic blood prothrombin was converted into thrombin at an abnormally slow rate This was in agreement with *Morawitz & Lossen* s (1908) demonstration of a higher concentration of thrombin in haemophilic serum than in normal serum an observation which they explained as due to presence of fewer or smaller amounts of anticoagulants in haemophilic serum

In 1947 *Quick* on the basis of Brinkhous observation developed his prothrombin consumption test The principle is that of determining the prothrombin time of the serum one or two hours after the blood has clotted This normally exceeds 40–60 seconds

The loss of prothrombin is a measure of its conversion into thrombin and consequently of the rate of thrombin formation

Slow conversion of prothrombin is seen in 1) deficiency of proaccelerin and proconvertin 2) platelet defects a) reduced number (thrombocytopenia) b) altered quality (thrombasthenia) 3) defects of the plasma thromboplastin (deficiency of AHF PTC PTA factor X or the Hageman factor) 4) inhibitors

If Quick s plasma prothrombin time is normal and presence of an inhibitor is excluded the result of the prothrombin consumption test indicates the thromboplastic activity of the plasma The clotting defect can then be classified more exactly by adding plasma samples with known clotting defects or specimens of known clotting factors to the tested plasma

If the serum prothrombin activity becomes low (60 seconds) by adding either AHF plasma Christmas plasma adsorbed normal plasma or normal serum the examined blood sample lacks PTA If it becomes normal after addition of AHF plasma or serum the tested plasma lacks the Christmas factor or PTA and if normalised after addition of Christmas plasma or adsorbed plasma the tested plasma lacks AHF or PTA Finally normalisation of the serum prothrombin activity after addition of heated adsorbed serum indicates deficiency of the Hageman factor

The prothrombin consumption test has been extensively used in the study of haemophilic blood having thus contributed essentially towards an understanding of the coagulation process (*Brinkhous Graham Penick & Langdell 1951 Dunn & Lyons 1951*) In a number of works the test has been used to differentiate between classical haemophilia (AHF deficiency) Christmas disease and Rosenthal s syndrome (*Aggeler White Glendening Page Leake & Bates 1952 Biggs Douglas Macfarlane Dacie Pitney Merskey & O Brien 1952 Schulman & Schmith 1952 van Creveld & Paulssen 1953 Frick 1954 Scardiagli & Guidi 1956 Rosenthal 1954*) Further the prothrombin consumption test has been used to diagnose combined haemophilia (*Hill & Speer 1955*) and finally to diagnose Hageman factor deficiency (*Frick & Hagen 1956 Ramot Singer Heller & Zimmermann 1956 Larrieu Soulier & Culot 1957*)

In the great majority of cases of haemophilia the prothrombin consumption test is suitable for demonstrating the clotting defect but it may fail in a few instances *Mond & Singer* (1951) have occasionally found a normal prothrombin consumption in haemophiliacs even in association with a prolonged clotting time *Brinkhous Langdell Penick Graham & Wagner* (1954) have described a patient with an antihaemophilic globulin concentration amounting to 16 per cent of the normal but with normal clotting time and prothrombin consumption *Biggs* (1955) has reported a case with an antihaemophilic globulin concentration of 7 5 per cent The prothrombin consumption was here very nearly normal Both patients led normal lives They had no haemarthroses but profuse haemorrhage after dental extractions

The prothrombin consumption test is hardly the ideal method of analysing haemophilic blood because patients may have a bleeding tendency without this method showing pathological conditions *Biggs* (1955) states that it will probably show normal conditions if the antihaemophilic globulin concentration exceeds 5 per cent of the normal The thromboplastin generation test as well as the thrombin generation test are more sensitive and likely to show abnormal thromboplastic conditions at antihaemophilic globulin concentrations up to 50 per cent of the normal (*Biggs 1955*)

Thrombin Generation Test

Like the two above mentioned methods of investigation the thrombin generation test differs from the remaining procedures of studying the coagulation in that it can be performed on blood without addition of foreign reagents

The principle of the thrombin generation test is that of transferring at one minute intervals a small amount of blood clotting at 37° C to a number of tubes containing fibrinogen solution The clotting time of the fibrinogen is determined in each tube This being approximately inversely proportional to the thrombin concentration (*Astrup & Darling 1942*) the reciprocal value for the clotting time of the fibrinogen specimen indicates the thrombin concentra

tion at the time concerned Provided the concentrations of prothrombin proconvertin and inhibitors in the blood sample tested are normal the thrombin formation will express the thromboplastic activity of the blood

As early as 1911 *Addis* by a thrombin generation test demonstrated a delayed thrombin generation in haemophilic blood This was borne out by *Hecht* (1942) who centrifuged the clotting blood and at intervals transferred five drops of the plasma serum mixture to fibrinogen solutions

Macfarlane & Biggs (1953) gave the thrombin generation test its present form They found a steep rise of the thrombin concentration after a lag period lasting 2 to 4 minutes Six minutes after the beginning of the test the thrombin concentration fell again Addition of small amounts of brain thromboplastin to the blood eliminated the lag period but the remaining course of the thrombin generation remained unchanged This was regarded as evidence to suggest that the blood thromboplastin had the same activity as brain thromboplastin A fall of the platelet count was accompanied by less thrombin formation The lag period was considerably prolonged in haemophilic blood (deficiency of anti haemophilic factor) Where the clotting time of haemophilic blood exceeded 30 minutes no thrombin whatever was formed Addition of small amounts of plasma or AHF to haemophilic blood effected normal thrombin formation

Pitney & Dacie (1953) employed *Macfarlane & Biggs* (1953) method on citrated plasma after recalcification and found delayed thrombin generation in plasma from haemophiliacs

The thrombin generation test has been used to a moderate extent only in the study of haemophilia *Astrup* (1954 not published) investigated one patient and *Gormsen* (1956) one *Sjølin* (1956) used the method to differentiate between classical haemophilia (AHF deficiency) and Christmas disease The same author has used the thrombin generation test on citrated plasma to investigate the Hageman trait (1957 b) as well as combined haemophilia (AHF and Christmas factor deficiency) (1957 c)

Regarding investigations of clotting defects reference may besides be made to *Stefanini & Dameshek* (1955) *Biggs & Macfarlane* (1957) and *Astrup & Sjølin* (1957) The technique of coagulation studies has been described in detail in the two former works

The *prothrombin consumption* test has been extensively used *in the study* of haemophilic blood having thus contributed essentially towards an under standing of the coagulation process (*Brinkhous Graham Penick & Langdell* 1951 *Dunn & Lyons* 1951) In a number of works the test has been used to differentiate between classical haemophilia (AHF deficiency) Christmas dis ease and Rosenthal s syndrome (*Aggeler White Glendening Page Leake & Bates* 1952 *Biggs Douglas Macfarlane Dacie Pitney Merskey & O Brien* 1952 *Schulman & Schmith* 1952 *van Creveld & Paulssen* 1953 *Frick* 1954 *Scardiagli & Guidi* 1956 *Rosenthal* 1954) Further the prothrombin consump tion test has been used to diagnose combined haemophilia (*Hill & Speer* 1955) and finally to diagnose Hageman factor deficiency (*Frick & Hagen* 1956 *Ramot Singer Heller & Zimmermann* 1956 *Larrieu Soulier & Culot* 1957)

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must be followed for many hours to record the complete prothrombin conversion

Another objection to the prothrombin consumption test is that the testing has to be started immediately after the sampling The blood sample cannot be transported over relatively long distances before the testing *Sjölin* (1956) as was necessary in the case of the present investigation

Using the thrombin generation test (*Macfarlane & Biggs* 1953 *Pitney & Dacie* 1953) the generated amount of thrombin is measured directly Even small amounts of thrombin are easily detected so accordingly it is possible to follow the initial phases of the thrombin generation during which the thrombin concentration rises rapidly The short course of testing makes it easier to keep the testing conditions constant Finally the method has the advantage that it can be used on citrated plasma

The thromboplastin generation test has been extensively used for classification of haemophilic diseases (*Biggs & Douglas* 1953 *Ramot Angelopoulus & Singer* 1955 *Verstraete & Vandenbroucke* 1955 *Verstraete* 1955 *Ratnoff & Margolius* jr 1955 *Iversen & Baastrup Madsen* 1956 *Ramot Singer Heller & Zimmerman* 1956 *Oehme & Hagette* 1955 *Miale & Wilson* 1956) This method requires a rather great preparatory work with preparation of platelet suspension and substrate plasma though the plasma suspension can be replaced by a brain extract (*Bell & Alton* 1954) Further it requires thrombin free serum and adsorbed plasma In the present study the thromboplastin generation test was used to a very limited extent only because it appeared that many of the mild defects were not demonstrable in this way (e g family 23 and family 88) *Biggs & Macfarlane* (1957) likewise found that this method might be inadequate as a diagnostic aid in mild cases of haemophilia

The thrombin generation test has the advantage over the thromboplastin generation test that one works with a system with fewer reagents (citrated plasma and calcium chloride) which therefore is easier to control (See also *Sjölin* 1956 *Astrup & Sjölin* 1957)

Qualitative Determination of Clotting Defects

In the first work concerned with a classification of haemophilic diseases in subgroups this was based on testing the mutual influences of haemophilic blood samples on their coagulability

It is however inappropriate to define one pathological condition by another in this case one clotting defect by another The testing will then depend on the availability of blood samples with definite clotting defects

Further it is a disadvantage that a series of tests are only reproducible by other workers if these are able to procure corresponding test plasmata and test sera (*Ramot Angelopoulus & Singer* 1955 a *Sjölin* 1957 c *Astrup & Sjölin* 1957)

Chapter IV

METHODS OF INVESTIGATION AND REAGENTS

It has been mentioned previously that the clotting defect in haemophilia is due to a defect in the thromboplastic system of the plasma (*Brinkhous* 1939 1947 *Quick* 1947) and that the components of the prothrombin complex are present in normal amounts (*Addis* 1911 *Quick* 1935 a b)

To study the qualitative clotting defect two principles of investigation are therefore required one to measure whether the composition of the prothrombin system is normal (prothrombin proaccelerin proconvertin), and one to make out about the composition of the plasma thromboplastin

Four methods are possible for evaluating the activity of plasma thromboplastin 1) clotting time determination 2) prothrombin consumption test 3) thromboplastin generation test and 4) thrombin generation test

Measurement of the Thromboplastic Activity
Choice of Method

Clotting time determination During performance of the thrombin generation test it is possible to determine the recalcification time in diluted plasma As stated previously this method is fairly insensitive, and normal times are often seen in haemophilia If employed alone clotting time determination would be insufficient for classification of the clotting defect

The prothrombin consumption test (*Brinkhous* 1939 *Quick* 1947) is a very frequently employed and very useful test in haemophilia It measures the loss of prothrombin and consequently its conversion into thrombin thus indirectly indicating the velocity of thrombin generation Accordingly this method can express the thromboplastic activity of the plasma provided the components of the prothrombin complex as measured by Quick s method are present in normal amounts and inhibitors are not present in abnormal concentrations Prothrombin is normally present in high concentrations in the blood On this account determination of minor falls of the prothrombin concentration as seen during the clotting of haemophilic blood will give inaccurate values these representing a small difference between two large amounts of prothrombin Hence complete or very pronounced conversion of prothrombin is essential for this method to be useful and in cases of clotting defects the coagulation process

after spontaneous clotting at 37 ° C The clotted blood was left for 4 to 6 hours at 37 ° C with the clot It was then stored over night in refrigerator (—4 ° C) The next day the clot was centrifuged off (2500 r p m for 15 minutes) Testing for remaining thrombin activity was performed by adding 0 1 ml of serum to 0 4 ml of 0 15 % fibrinogen solution at 37° C If no clot occurred within 45 minutes the serum was regarded as thrombin free and was stored at —20 ° C

Readsorbed serum A portion of the above mentioned serum was adsorbed twice with 20 mg $BaSO_4$ per millilitre of serum for 30 minutes The barium sulphate was removed after each adsorption by centrifugation (2500 r p m for 10 minutes)

Heated readsorbed serum was prepared from the above readsorbed serum by heating it to 56 ° C for 30 minutes in water bath

Platelet suspension was prepared from citrated plasma which was centrifuged for 2 minutes (1000 r p m) The pipetted plasma (silicone treated pasteur pipettes) was centrifuged at 2500 r p m for 20 minutes in siliconed tubes The deposited platelets were washed four times in physiological saline and then resuspended in saline The platelets were counted in Burker Turk s counting chamber Freshly prepared platelet suspensions as well as suspen sions stored at —20 ° C were used

All the plasma and serum samples were stored at —20 ° C Some plasma samples were stored with their normal platelet content (platelet rich plasma) and some after centrifugation for 15 minutes 2500 r p m (platelet poor plasma) If the number of platelets in the platelet poor plasma was not under 10 000 per cubic millimetre the plasma was recentrifuged

The tubes and pipettes were cleaned in sodium carbonate solution and chromium sulphate and next rinsed six times with tap water and once with distilled water The tubes were dried in incubator The tubes for blood sam pling and the pasteur pipettes were *silicone treated* with dimethyldichlorosilane (Corning corporation) After rinsing and drying the tubes were placed for about 3–4 hours in a closed glass container in which there was a small tube with a few millilitres of dimethyldichlorosilane

Cannulae 4 cm long caliber 11 dry sterilised not silicone treated

Methods

Thrombin Generation Test

4 5 ml of blood were passed direct by venous puncture (clean entering of the vein) into a silicone treated tube containing 0 5 ml of 3 8 % citrate solution The blood and the citrate were further mixed by cautiously turning the tube upside down twice The blood samples were placed in ice-cold water until the testing The citrated blood was centrifuged for 2 minutes (1000 r p m)

Using a constriction pipette (Carlsberg) 1 ml of plasma was pipetted over

Finally if haemophilic plasma is used as test substrate this must be stored frozen This is a drawback because we know that freezing may alter the coagulability of haemophilic blood (*Govaerts & Gratia* 1931 *Sjølin* 1956 b)

Adsorbed bovine plasma normal serum heated adsorbed serum and platelet suspensions which anybody can prepare have therefore been chosen as reagents for defining the clotting defects in haemophilia These tests have in some instances been supplemented by studies on the influence of freezing on the plasma as well as by cross tests between different haemophilic plasmata and between haemophilic plasmata and haemophilic sera The latter have been performed as a kind of control as it proved impossible in cases of mild defects to manage with classification on the basis of normal reagents alone

Addition of human citrated plasma has also been employed as a means of testing for the presence of inhibitors Inability of normal plasma to correct the clotting defect suggests presence of an inhibitor

The added amounts of reagents generally constitute about 20 per cent of the plasma volume to be tested i e 0 2 ml of reagent to 1 ml of plasma in rare cases 30–40 per cent

A review of the literature as well as experiments with addition of varying amounts of reagents showed this ratio to be suitable in most cases

Macfarlane & Biggs (1953) in experiments using the thrombin generation test found that 10 per cent of normal plasma is unable to normalise the thrombin generation in haemophilic blood According to *Pitney & Dacie* (1953) about 20 per cent or more of normal plasma is necessary to render the thrombin generation normal These quantities are large compared with those stated by *Patek & Stetson* (1936) The latter workers found that addition of 1/40 vol % of citrated blood and even as little as 1/200 vol % could give optimum shortening of the clotting time in haemophilic blood

Reagents

Saline 0 85 % NaCl

Sodium citrate $C_6H_5O_7Na_3$ 2H O 3 8 % in water

Calcium chloride 0 025 M CaCl in aqueous solution

Saline buffer Sodium diethylbarbiturate buffer (Michaelis) 0 05 M pH 7 8 containing 0 10 M NaCl *Total* μ = *0 15*

Adsorbed bovine plasma Adsorbed twice with 20 mg $BaSO_4$ (Pharmacopea Danica 1948) per millilitre for 30 minutes (*Brodthagen* 1953)

Bovine fibrinogen (*Astrup & Mullertz* 1952) diluted with saline buffer immediately before use to 0 15 % fibrinogen A stock solution of fibrinogen was prepared once a week

Human brain thromboplastin was prepared according to *Owren* (1949)

Serum to be used as sources of Christmas factor plasma thromboplastin antecedent Hageman factor and factor VII was prepared from whole blood

after spontaneous clotting at 37 ° C The clotted blood was left for 4 to 6 hours at 37 ° C with the clot It was then stored over night in refrigerator (—4 ° C) The next day the clot was centrifuged off (2500 r p m for 15 minutes) Testing for remaining thrombin activity was performed by adding 0 1 ml of serum to 0 4 ml of 0 15 % fibrinogen solution at 37° C If no clot occurred within 45 minutes the serum was regarded as thrombin free and was stored at —20 ° C

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Using a constriction pipette (Carlsberg) 1 ml of plasma was pipetted over

into a tube and mixed with 1 ml of saline The mixture was placed in a water bath (37 ° C) To each of 13 uniformly calibrated dwarf tubes (internal diameter 8 5 mm) placed in the water bath was added 0 4 ml of 0 15 % fibrinogen solution Then the plasma saline mixture was recalcified with 1 ml of M/40 calcium chloride solution (37 ° C) The contents of the tube were mixed carefully and a stop-watch was started One minute after the recalcification and thereafter at intervals of one minute 0 1 ml of the clotting plasma mixture was transferred by micropipette to successive fibrinogen tubes The clotting times for the fibrinogen solutions (t) were recorded At a certain point of time the recalcified plasma mixture would clot This calcium clotting time or recalcification time was recorded The clot formed was pressed against the wall of the tube with two wooden sticks to squeeze out any serum left For each test a curve was plotted expressing the ratio between the clotting time of the fibrinogen solution (t) and the reaction time of the plasma sample (T) To be able to compare the results of the tests an arbitrary thrombin unit has been chosen which is the reciprocal value of the clotting time t of the fibrinogen solution measured in seconds and calculated as 600/t As mentioned previously the thrombin concentration is inversely proportional to the clotting time of fibrinogen (*Astrup & Darling* 1942) By way of control samples of fresh normal bank blood were submitted daily to thrombin generation test

The clotting defects were classified according to the scheme shown in fig 6 where + indicates that the thromboplastic activity in the patient s plasma became normal after addition of 0 2 ml of one of the reagents in the column to the left The bottom line illustrates the influence of freezing on the thromboplastic activity

Prothrombin Time

All the blood samples were submitted to prothrombin time determination according to Quick using human brain thromboplastin (*Biggs & Macfarlane* 1953)

Fig 6

The patient s plasma lacks

Added reagent	Antihaemophilic factor (classical haemophilia)	Christmas factor (Christmas disease)	Plasma thromboplastin antecedent (Rosenthal's syndrome)	Hageman factor (Hageman's trait)
adsorbed bovine plasma	+	0	+	+
normal serum	0	+	+	+
heated readsorbed serum	0	0	0	+
freezing of platelet rich plasma	(0)	(0)	+	+

Further prothrombin proconvertin determination was made in a number of cases using Owren s P P method (*Owren* 1949 *Astrup Mullertz & Hansen* 1951)

Platelet Count

Thrombocyte counts were undertaken in undiluted citrated plasma (one part of 3 8 % sodium citrate to nine parts of venous blood) after a few hours of spontaneous sedimentation or after centrifugation for 2 minutes (1000 r p m) The counts were made in Burker Turk s counting chamber with half chamber height (0 05 mm) The thrombocyte values are stated per µl of plasma

Chapter V

THE NORMAL THROMBIN GENERATION TEST

As mentioned previously thrombin generation tests were performed daily on one or two plasma samples originating from apparently healthy donors to control the usefulness of the fibrinogen solution

Fig 7 shows some curves for the thrombin concentration plotted against the time It is seen that after a lag period of 1–4 minutes during which the plasma thromboplastin is activated a rather steep rise of the thrombin concentration follows which reaches maximum in the course of 4–7 minutes This part of the *curve expresses the thromboplastic activity* of the plasma Then a fall occurs in the thrombin concentration This fall generally takes place at a slower rate than the preceding rise The inactivation of the thrombin which starts immediately on its formation and which is due partly to adsorption on the formed fibrin (*Quick & Favre Gilly* 1949) *and partly to an antithrombin action* (*Astrup & Darling* 1942 1943) is generally finished after 10–13 minutes

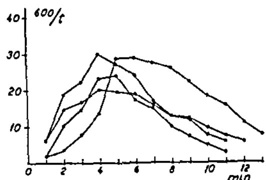
Variations in the Course of the Thrombin Generation in the Control Material

Investigations of the course of the thrombin generation in the control material revealed as stated very great variations in the normal thrombin generation (see fig 7) It is of no interest to render all the control curves (about 1000) and tables recording the variations of the thrombin concentrations would be confusing

Idealized curves for the course of the thrombin generation have therefore been plotted by drawing straight lines through the ascending and descending sections of the curves The points at which these lines intersect the abscissa mark the beginning and conclusion of the thrombin generation respectively provided the generation and inactivation proceed at a constant velocity (see fig 8)

Four points have been chosen to describe these curves 1) *the time of* beginning thrombin generation 2) the moment of maximum thrombin concentration 3) the time of total thrombin inactivation and 4) the maximum thrombin concentration obtained (600/t)

These calculations were based on 107 unselected control curves among the normal curves



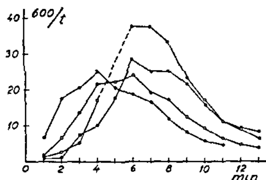


Fig 7

Thrombin generation in eight normal plasma samples
Abscissa Reaction time (T) in minutes
Ordinate Reciprocal values of the clotting times for the fibrinogen solution expressed as 600/t (t in seconds)

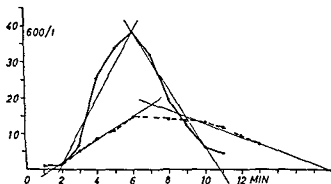


Fig 8

Thrombin generation in two normal plasma samples Straight lines have been drawn through the ascending and descending parts of the curves (see text) Abscissa and ordinate as in fig 7

1 *The Time of Beginning Thrombin Generation*

Fig 9 illustrates in the form of a dot diagram the distribution of 107 times of beginning thrombin generation The distribution (cumulated frequency curve) is judged to be normal

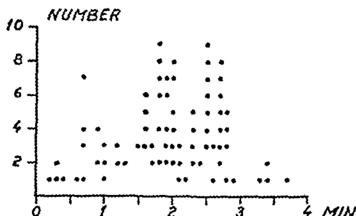


Fig 9

Times of beginning thrombin generation.
Abscissa Beginning of thrombin generation
Ordinate Number of samples

The mean of the observations is 1 89 The standard deviation has been calculated according to the formula

$S = \sqrt{\dfrac{\varepsilon (t^2) - (\varepsilon t)^2/n}{n-1}}$ (*Kemp* 1955) where t represents the single observations and n the number of observations

$S = \sqrt{\dfrac{434 - 40844\ 41/107}{106}} = 0\ 7$ Standard error $= 0\ 07$

Mean $= 1\ 89 \pm 0\ 07$

The time of beginning thrombin generation lies within $1\ 89 \pm 0\ 7 \times 3$ i e 0–4 minutes

2 *The Moment of Maximum Thrombin Concentration*

The dot diagram fig 10 shows the distribution of 107 observations The distribution is judged to be normal

Standard deviation $S = \sqrt{\dfrac{3519\ 76 - 364332\ 96/107}{106}} = 1\ 04$

Standard error $= 0\ 101$

Mean $= 5\ 64 \pm 0\ 101$

The moments of maximum thrombin concentration lie within $5\ 64 \pm 1\ 04 \times 3$ i e between about 2 5 and 8 7 minutes

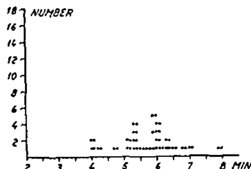


Fig 10

The moments of maximum thrombin concentration
Abscissa Moments of maximum thrombin generation.
Ordinate Number of samples

In a control material of 15 plasma samples *Pitney & Dacie* (1953) found maximum thrombin concentration between 2 5 and 4 5 minutes after the recal cification

3 *The Time of Total Thrombin Inactivation*

The dot diagram fig 11 shows the distribution of 107 observations The distribution is judged to be normal

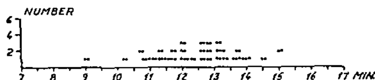


Fig 11

The times of concluded thrombin generation
Abscissa Times of concluded thrombin generation
Ordinate Number of samples

Standard deviation $S = \sqrt{\dfrac{15962\ 51 - 1662294\ 49/107}{106}} = 2\ 007$

Standard error = 0 19

Mean = 12 05 ± 0 19

The times of total thrombin inactivation lie within 12 05 ± 2 01 × 3 i e be tween 6 and 18 minutes after the recalcification *Pitney & Dacie* (1953) found that all the thrombin had been inactivated about 10 minutes after the recalcification

4 *The Maximum Thrombin Concentration Obtained*

The dot diagram fig 12, shows the distribution of 107 observations The distribution is normal

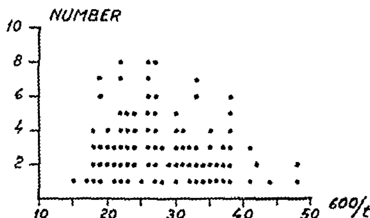


Fig 12

The maximum thrombin concentrations obtained
Abscissa Maximum thrombin concentrations determined as 600 t
Ordinate Number of samples

$$\text{Standard deviation } S = \sqrt{\frac{89922\,5 - 84168\,2}{106}} = 7\,369$$

Standard error = 0 7123

Mean = 28 05 ± 0 71

The maximum thrombin concentrations lie within 28 05 ± 7 4 × 3 i e between 50 and 6 arbitrary units

It is plain to see that each of the four values for the normal thrombin generation show considerable variations specially that for the maximum throm bin concentration This must be due in some measure to differences in the composition of the fibrinogen solutions

Moreover variations in the thrombin generation are referable to individual differences of the thromboplastic activity as well as to the technique of blood sampling

On reviewing the curves it appeared that the lag period the moment of maximum thrombin concentration and the time of completed inactivation do not vary independently A long lag period is generally accompanied by late attainment of maximum concentration and of total thrombin inactivation These curves are also usually rather low

Recalcification Times in the Control Material

The dot diagram fig 13 shows the distribution of 176 likewise unselected recalcification times in the control material

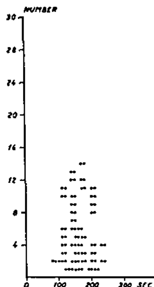


Fig 13

The distribution of 176 recalcification times from control material
Abscissa Recalcification times measured in seconds
Ordinate Number of samples

The mean of the observations is 153 sec

$$\text{Standard deviation } S = \sqrt{\frac{4431256 - \frac{(26931)^2}{176}}{175}} = 42\ 18$$

$$\text{Standard error} = \frac{42\ 18}{\sqrt{176}} = 3\ 18$$

Mean = 153 sec $\pm$ 3 18

Only 0 3 per cent of the recalcification times in a control material exceed 153 sec + 3 $\times$ 42 18 = 280 sec $\sim$ 4 min 40 sec

Repeated Investigations of the Thrombin Generation in the Same Subject

The thrombin generation was investigated several times in blood samples from the same subject for the purpose of throwing light on the problem whether the intra individual thromboplastic activity is constant Fig 14 shows the thrombin generation in citrated plasma from a man aged 35 with no signs of a haemorrhagic diathesis The samples were withdrawn at intervals of 3 months The lag period varied from about 4 (curve a) to about 2 minutes (curve b) The thrombin concentration was maximal after between 6 and 9 minutes Platelet

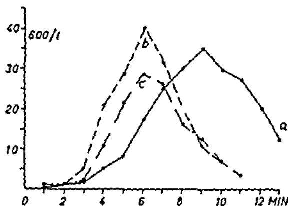


Fig 14

Repeated investigations of the thrombin generation in the same subject Abscissa and ordinate as in fig 7

counts in the three plasma samples a b c, gave 620 000 554 000 and 339 000 respectively The prothrombin time was normal

The lag period as well as the moment of maximum thrombin concentration thus seem to vary but little in the individual subject This is probably accountable for by little spontaneous variation in the thromboplastic activity of the plasma and/or small variations in the fibrinogen used

Biggs & Macfarlane (1953) found that different fibrinogen preparations made in the same way give different thrombin times with the same thrombin It was therefore natural to believe that variations of the curves were due to variations of the fibrinogen However if so we should in the first instance expect the height of the curves to vary and not the lag periods The slight differences demonstrated of the peaks may however be due to varying numbers of platelets the thrombin concentration rising with increasing numbers (*Biggs & Macfarlane* 1953) (see p 57)

In the three above mentioned tests the recalcification times were 4 min 45 sec 2 min and 3 min 30 sec respectively The recalcification times followed the corresponding lag periods about 4 min 1 min and 2 min This likewise argues in favour of the view that variations in the thromboplastic activity of plasma are responsible for the differing curves rather than variations of the fibrinogen

Repeated Investigations of the Thrombin Generation in the Same Plasma Sample

Fig 15 shows the results of ten thrombin generation tests on the same plasma sample The values found lie within the hatched area with only a small variability of observations

The simultaneously determined recalcification times ranged between 108 and 115 seconds

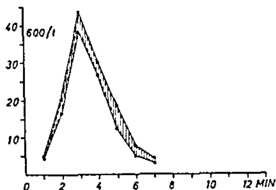
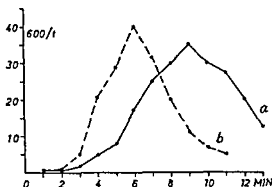


Fig 15

The course of the thrombin generation based on ten tests on the same plasma sample Abscissa and ordinate as in fig 7



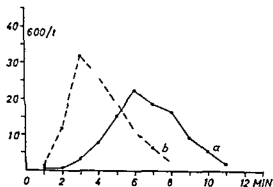


Fig 16 I and II

The influence of cooling down (+4 C) on the thrombin generation Curves (a) before the cooling down curves (b) after Abscissa and ordinate as in fig 7

Influence of Cooling Down on the Thrombin Generation

It is a well known fact that storage greatly improves the coagulability of haemophilic blood (*Brinkhous* 1939 *Quick* 1941 *Tocantins* 1942–1943 *Pavlovsky* 1947 *Graham Buchwalter Hartley & Brinkhous* 1949) *Pitney & Dacie* (1953) found that storage of the plasma for a few hours at + 4° C had no influence on the thrombin generation

In a few cases the thrombin generation in normal plasma was investigated after storage in refrigerator (+ 4° C) for about 24 hours Fig 16 I and II show the results of two such experiments Curves (a) illustrate the thrombin generation before the storage and curves (b) the generation after storage at + 4° C In both cases storage effected shortening of the lag period and a rise of the maximum thrombin concentration obtained The recalcification times were shortened in both cases Before the storage they were 4 min 45 sec and 3 min and after the storage 3 min 45 sec and 1 min 45 sec

Storage of normal plasma at + 4° C thus seems to raise the thromboplastic activity It is doubtful how this phenomenon is to be explained *Astrup* (personal communication) concludes from the results of *Nilsson & Wenckert's* (1954) experiments that it may be due to the degenerating platelets inactivating normally occurring inhibitors thus stimulating the process of activation

Influence of Freezing on the Thrombin Generation in Normal Plasma

Pitney & Dacie (1953) state that the thrombin generation proceeds more quickly in frozen recalcified plasma than in normal plasma and that the thrombin concentrations obtained are higher

Fig 17 illustrates the thrombin generation in fresh plasma (curve a) and in plasma stored for about 24 hours at –20° C (curve b) Curve b shows about four times faster rise of the thrombin concentration and a higher maximum concentration Also a fall of the recalcification time was found from 120 to 80 seconds The demonstrated improvement of the thrombin generation was presumably due to destruction of platelets brought about by the freezing and subsequent thawing the accelerating effect of freezing on the thrombin generation being only slight in thrombopenic blood Fig 18 a and b shows the course of the thrombin generation in plasma from a patient with thrombopenia (85 000 platelets per μl of plasma) before and after freezing The thrombin generation is seen to have improved only slightly after the freezing

Influence of the Thrombocytes on the Thrombin Generation

The number of platelets is known to influence the amount of thrombin formed (*Macfarlane & Biggs* 1953 *Pitney & Dacie* 1953) and to a smaller extent on the time of beginning thrombin generation (*Macfarlane & Biggs* 1953)

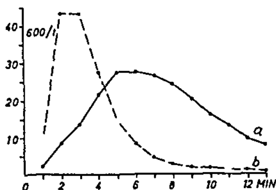


Fig 17

The influence of freezing on the thrombin generation (a) before freezing and (b) after freezing of the plasma Abscissa and ordinate as in fig 7

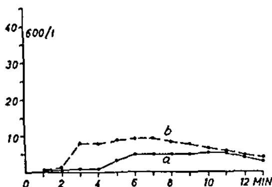


Fig 18

The influence of freezing on the thrombin generation in plasma from a patient with thrombopenia (a) before freezing and (b) after freezing of the plasma Abscissa and ordinate as in fig 7

Fig 19 (a) shows the thrombin generation in a normal plasma The number of thrombocytes was about 336 000 per μl of plasma Fig 19 (b) shows the thrombin generation in the same plasma after addition of 0 5 ml of platelet suspension (304 000 platelets per μl) to 1 ml of plasma The thrombin generation was greatly accelerated and the maximum concentration obtained was raised The recalcification times were 2 min 21 sec and 2 min 10 sec respectively

Similar results have been observed by investigating blood from patients with thrombopenia Fig 20 (a) shows the thrombin generation in plasma from a patient with thrombopenia (4000 platelets per μl of plasma) Addition of 0 4 ml of platelet suspension (380 000 platelets per μl) very soon gave rise to formation and inactivation of thrombin (fig 20 b) which reached a higher concentration The recalcification times were 3 min 40 sec and 1 min 45 sec respectively

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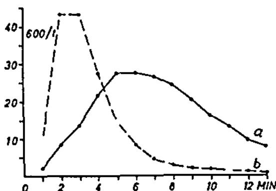


Fig 17

The influence of freezing on the thrombin generation (a) before freezing and (b) after freezing of the plasma Abscissa and ordinate as in fig 7

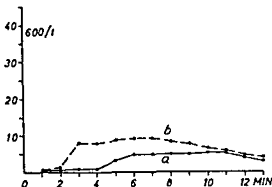


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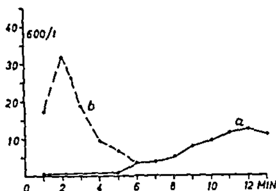


Fig 21

The thrombin generation in plasma from a patient with aplastic anaemia (a) before and (b) after addition of platelets Abscissa and ordinate as in fig 7

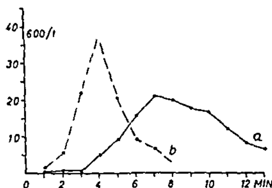


Fig 22

The thrombin generation in normal plasma (a) before and (b) after addition of heated readsorbed normal serum Abscissa and ordinate as in fig 7

Influence of Serum on the Normal Thrombin Generation

O Brien (1955) found serum to have a platelet like activity as it shortened the clotting time and accelerated the thrombin generation when added to platelet rich plasma The platelet like factor was stable for one hour at 56° C

Fig 22 (a) shows the thrombin generation in normal plasma Addition of 0 2 ml of heated readsorbed serum to 1 ml of this plasma raised the velocity of thrombin generation as well as the maximum thrombin concentration (fig 22 b)

The same was seen using untreated serum (fig 23)

Addition of heated readsorbed normal serum to plasma from a patient with thrombopenia likewise stimulates the thrombin generation Fig 24 (a) shows

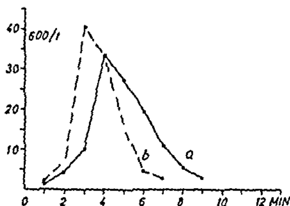


Fig 19

The course of the thrombin generation in plasma (a) before and (b) after addition of platelets Abscissa and ordinate as in fig 7

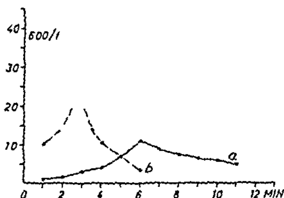


Fig 20

The thrombin generation in plasma from a patient with thrombopenia (a) before and (b) after addition of platelets Abscissa and ordinate as in fig 7

Fig 21 (a) illustrates the thrombin generation in plasma from a patient with aplastic anaemia Thrombocyte count 5000 per µl plasma Addition of 1 ml of platelet suspension (119 000 platelets per µl) very soon gave a rise and a fall of the thrombin concentration which reached a very high maximum (Fig 21 b) At the same time the recalcification time fell from 6 min 5 sec to 1 min

An increased number of platelets thus seems to accelerate the thrombin generation and to raise the thrombin concentration

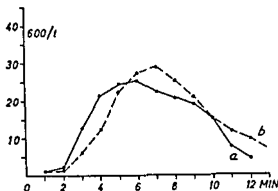


Fig 25

The course of the thrombin generation in normal plasma (a) before and (b) after addition of adsorbed bovine plasma Abscissa and ordinate as in fig 7

Influence of Adsorbed Bovine Plasma on the Thrombin Generation

Fig 25 (a) and (b) illustrates the course of the thrombin generation in 1 ml of normal plasma before and after addition of 0 2 ml of adsorbed bovine plasma The thrombin concentration seems to have been slightly increased but no acceleration of the coagulation process was seen

Influence of Washed Platelets on the Thrombin Generation

Fig 26(a) shows the thrombin generation in a control plasma containing 244 000 platelets per cubic millimetre Fig 26 (b) shows the thrombin generation in the same plasma after the platelets had been removed washed four times in saline and re added to the plasma from which they had been isolated

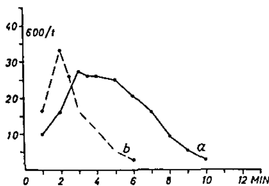


Fig 26

The thrombin generation in normal plasma (a) before and (b) after its platelets had been washed in physiological saline Abscissa and ordinate as in fig 7

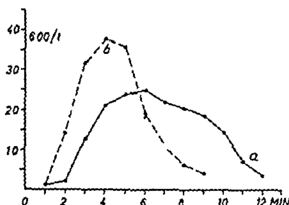


Fig 23

The course of the thrombin generation in normal plasma (a) before and (b) after addition of normal serum Abscissa and ordinate as in fig 7

the thrombin generation in a plasma containing 85 000 thrombocytes per cubic millimetre of plasma Addition of 0 2 ml of heated readsorbed serum to 1 ml of this plasma greatly accelerated the generation of thrombin which reached high concentrations (fig 24 b) A similar effect was obtained by adding 0 4 ml of platelet suspension (187 000 platelets per cubic millimetre) (fig 24 c) The recalcification times in the three experiments were 5 min 40 sec 3 min and 1 min 55 sec respectively

These experiments confirmed that serum contains a factor (or factors) having a platelet like effect It tolerates heating to 56° C and is not removed by adsorption with barium sulphate

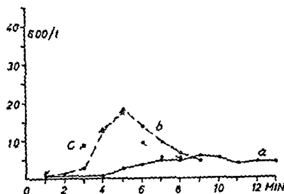


Fig 24

The course of the thrombin generation in platelet poor plasma (b) after addition of heated readsorbed serum (c) after addition of platelets Abscissa and ordinate as in fig 7

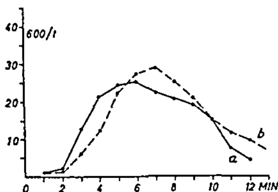


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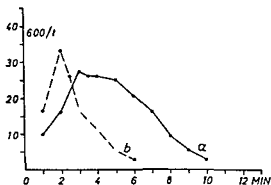


Fig 26

The thrombin generation in normal plasma (a) before and (b) after its platelets had been washed in physiological saline Abscissa and ordinate as in fig. 7

The curve shows a very considerable acceleration of the thrombin generation after the washing The recalcification time was 60 seconds in both experiments

Prothrombin Complex and Thrombin Generation

Whereas there has been no occasion to investigate plasma from patients with congenital defects of the prothrombin complex the thrombin generation has been studied in plasma from various patients during dicoumarol treatment Dicoumarol has long been known (*Link* 1944) to cause Hypoprothrombinaemia It has since been shown that in addition to diminishing the prothrombin content dicoumarol reduces the contents of factor VII (*Owen & Bollman* 1948 *Owren & Aas* 1951) factor X (*Koller* 1955) the Stuart Prower factor (*Bachman Duckert Geiger Baer & Koller* 1957) and though of less interest in this connection the Christmas factor (*Verstraete* 1955 *Sise Kimball & Adams* 1955 *Johnson Seegers Koppel & Olwin* 1957)

Fig 27 shows the course of the thrombin generation in a patient during dicoumarol treatment Curve (a) represents the thrombin generation at a time when the prothrombin proconvertin level was 69 per cent measured according to Owren The curve runs a perfectly normal course At the time of the experiment illustrated in curve (b) the p-p level was 15 per cent Curve (c) corresponds to a p-p value of 7 4 per cent It is plain to see that a pronounced fall of the p-p level is accompanied by a decreasing thrombin generation

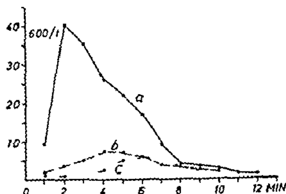


Fig 27

The course of the thrombin generation in plasma from a patient under dicoumarol treatment
a p-p acc to Owren 69 / b p-p 15 /
c p-p 7 4
Abscissa and ordinate as in fig 7

Chapter VI

COLLECTION OF MATERIAL

Introduction

Regarding the geography and population of Denmark the following informa tion will be given below

The kingdom of Denmark comprises Denmark proper situated between 55 and 58 degrees N and between 8 and 15 degrees E L of Grw as well as Greenland and The Faroe Islands

The present investigation of haemophiliacs comprises solely those living in Denmark proper i e the peninsula of Jutland bordering in the South on Germany and the islands of Seeland with the Capital of Copenhagen on the East as well as Funen Lolland Falster Bornholm and a number of smaller islands

The total area of Denmark is about 43 000 square kilometers The popula tion (Danish Year Book of Statistics 1956) numbers 4 281 275 fairly equally distributed between rural and urban districts

The population belongs in the main to the Nordic race being tall (160–180 cm) fair dolichocephalic or subdolichocephalic and often blue-eyed A great deal of immigration has taken place in the course of time especially from the South and from Sweden

The Families Investigated

A series of 148 patients distributed over 78 families have had their coagula tion system investigated Seventy five of these patients belong to 35 of the 67 fa milies included in *Andreassen* s (1943) series However of these 75 patients no more than 35 were described by *Andreassen* The remaining 40 were born after Andreassen had collected his material In 32 of *Andreassen* s 67 families there were no living haemophiliacs by the time the present investigation took place The data of the majority of the remaining 73 patients were obtained from Danish provincial hospitals

In addition to the 148 patients investigated the author knows of another eight haemophiliacs who either refused to co operate (three) or were too young for blood sampling (three) or who had emigrated (two)

Prevalence

Andreassen (1943) found 81 haemophiliacs in a population of 3 844,312 i e about one haemophiliac per 50 000 inhabitants or about one haemophiliac per 23,000 male inhabitants

The author of the present work found 156 haemophiliacs in a population of 4 281 275 (Danish Year Book of Statistics 1956) This gives about one haemophiliac per 27 000 or about one per 14 000 males

The literature contains only few statements of the incidence of haemophilia According to *Hecht* (1955) about 300 bleeders are known in Holland, but the actual number is probably about 600 This corresponds to about one per 10 000 inhabitants *Verstraete* (1955), who has described 42 cases of haemophilia, is of the opinion that the incidence of classical haemophilia (AHF deficiency) is about one per 10 000 and that of Christmas disease (PTC deficiency) one per 100 000 *Biggs & Macfarlane* (1953) believe that haemophilia affects roughly one or two persons per 100 000 on the British Isles

Comparing the Danish series from 1943 with the present it seems as if the incidence of haemophilia has risen considerably It appears however that 28 of the haemophiliacs in the present series who had been born before 1942 are not included in the 1943 series Correction of *Andreassen*'s figures gives an incidence of haemophilia of about one per 35 000 or about one per 17 000 male inhabitants

The incidence of haemophilia thus seems to have increased only moderately The question whether all haemophiliacs in Denmark have been registered must be answered in the negative Some have such slight symptoms that they lead a perfectly normal life having never consulted a doctor on account of the disease In such cases haemophilia is often discovered accidentally e g on dental extraction (See for instance family 51, p 252 and family 109 p 334) After the conclusion of the present investigation the author found yet another patient with classical haemophilia and one with the Hageman trait

Pedigrees

The pedigrees of the haemophilic families are rendered in the last chapter of the book The first 35 pedigrees are identical with those having the same numbers in *Andreassen*'s work They have been brought up to date

They comprise those of Andreassen's haemophilic families in which haemophiliacs were alive and examined by the author at the time of the investigation

The remaining 43 pedigrees have been drawn on the basis of personal communications obtained from the living members of the families Where possible each pedigree comprised not less than four generations

Case Records

A case record has been made for each of the living haemophiliacs including information on the bleeding tendency These records are based on personal questioning and examinations of all the patients except six For patients who have been in hospital the hospital records have been reviewed Possible information in these on the conditions of coagulation is rendered in the case records

A point was made of procuring infomation on the first manifestation of the disease the nature of the bleeding (bleeding in the skin the muscles the nose the joints the gums haematemesis melaena haematuria) the changes in signs and symptoms with increasing age and surgical interventions if any Finally information has been procured as to how the patients manage in life Along with the case reports which are rendered below the respective pedigrees and which have consecutive numbers within each family the most important haematological examinations are stated

Collection of Blood Samples

Under the existing circumstances it was found most expedient to perform the samplings and examinations of the patients in their respective homes The investigations took place during week ends On Saturday afternoon the part of the country to be searched for haemophiliacs was visited In the course of Sunday the haemophiliacs usually three or four were looked up and blood samples were withdrawn These were placed in a cooler containing ice water and transported to the laboratory where they were analysed the next day The blood samples originating from patients outside Seeland were thus stored at 0° C for about 18–20 hours before the analysis

Influence of Storing on the Thromboplastic Activity of Haemophilic Blood

It is a well known fact that the coagulability of haemophilic blood is increased by standing at room temperature (*Brinkhous* 1939 *Quick* 1941 *Pavlovsky* 1947) No satisfactory explanation can be given of this phenomenon *Brinkhous* (1939) and *Quick* (1941) supposed it to be due to disintegration of platelets and *Pavlovsky* (1947) to inactivation of an inhibitor the decreasing clotting time being independent of the presence of platelets

The author has tested the thromboplastic activity of a few samples of haemophilic blood before and after storage at 0° C for 24 hours

Fig 28 curve A shows the thrombin generation in plasma from a patient with AHF deficiency (family 68 no 3) The recalcification time was 15 minutes Curve B shows the thrombin generation after the blood sample had been stored at 0° C for 24 hours The thrombin generation had not improved and the recalcification time was 14 min 30 sec

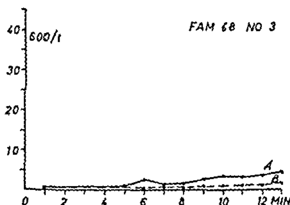


Fig 28

The thrombin generation in haemophilic plasma (AHF deficiency) (a) before and (b) after storage at 0 C for 24 hours Abscissa and ordinate as in fig 7

Fig 29 illustrates corresponding experiments on patient no 1, family 108 (AHF deficiency + serum factor deficiency) The recalcification times were 6 min and 5 min 40 sec respectively There was no significant difference be tween the thrombin generations in the two experiments

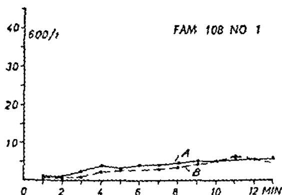


Fig 29

Courses of the thrombin generation in haemophilic plasma (AHF deficiency + serum factor deficiency) (a) before and (b) after storage at 0 C for 24 hours Abscissa and ordinate as in fig 7

Fig 30 shows the thrombin generation in plasma from a patient with Christmas disease (family 62 no 3) The curves are identical There is no difference between the thrombin generations before and after storage in ice water for 24 hours

On the other hand spontaneous improvement of the thrombin generation seems to be possible in plasma from patients lacking the Hageman factor In plasma from patient no 2 family 112 (see p 339) the thrombin generation

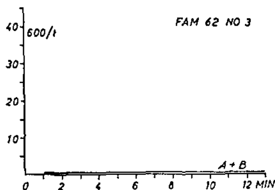


Fig 30

The thrombin generation in haemophilic plasma (deficiency of the Christmas factor) (a) before and (b) after storage at 0 C for 24 hours Abscissa and ordinate as in fig 7

became normal after storage in ice water for 24 hours in a siliconed glass tube There was a slight fall in the recalcification time from 5 min 30 sec to 4 min 50 sec

Fig 31 curve A shows the thrombin generation in plasma from a patient with a mild antihaemophilic factor deficiency (family 31 no 6) The recalcification time was 6 min 50 sec Curve B shows the thrombin generation in plasma stored at 0° C for 24 hours Here too the thrombin generation was abnormal though somewhat accelerated just as also the recalcification time had fallen to 5 min

The results of the above experiments go to show that it is possible to investigate the coagulation system in blood from patients with AHF deficiency and Christmas factor deficiency after the blood has been stored at 0° C for

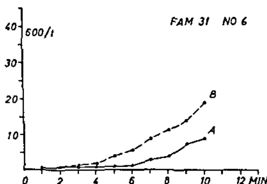


Fig 31

The thrombin generation in haemophilic plasma (mild AHF deficiency) (a) before and (b) after storage at 0 C for 24 hours Abscissa and ordinate as in fig 7

24 hours as storage seems to cause no appreciable changes of the clotting defect Difficulties may arise if the blood sample stored in this way is derived from a patient lacking the Hageman factor or a patient with a mild defect However the author has had occasion to examine blood samples from most of these patients several times partly as usual in Copenhagen and partly in a local laboratory, where the blood coagulation system could be tested In no instance was there found a normal thromboplastic activity in plasma from a patient with persistent signs and symptoms of a haemorrhagic diathesis

Chapter VII

DIAGNOSIS AND SYMPTOMATOLOGY OF HAEMOPHILIC DISEASES

Diagnosis

In the pronounced cases of haemophilia the diagnosis generally causes little difficulty Haemophilia is a haemorrhagic diathesis due to a defect of the plasma thromboplastin The classical form of haemophilia is a hereditary disease with sex linked recessive transmission (*Nasse* 1820 *Schloessmann* 1924) In about 30 per cent of the cases only one patient can be found in each family (*Andreassen* 1943 *Dahlberg* 1949 *Stefanini* & *Dameshek* 1955) These represent the so-called sporadic haemophilia which is indistinguishable from the hereditary form both clinically haematologically and genetically The disease occurs almost exclusively in males

Differential Diagnoses

Haemophilia is characterised haematologically by 1) most often a prolonged clotting time 2) a delayed conversion of prothrombin into thrombin owing to deficient formation of plasma thromboplastin The prothrombin time the content of platelets and the bleeding time are normal

To give a brief survey of the most important problems of differential diagnosis in cases of haemorrhagic diathesis it is expedient to divide these in 1) the plasmatically induced 2) the thrombocytogenic and 3) the vascularly induced

Plasmatically Induced Haemorrhagic Diatheses

Haemophilia (1) belongs as stated to the plasmatically induced haemorrhagic diatheses This group includes further 2) Hypoprothrombinaemia hypoproaccelerinaemia and hypoproconvertinaemia These diseases are characterised by a prolonged clotting time as well as by a prolonged Quick s prothrombin time 3) Diseases with a disturbed fibrin formation Afibrinogenaemia and fibrinogenopenia In the former no clot is formed by adding thrombin and in the latter a prolonged clotting time and decreased fibrin formation are seen

The plasmatically induced clotting defects mentioned under 2) and 3) may

be congenital or acquired The congenital defects may be familial but nothing certain is known as yet of the mode of transmission

Finally, 4) conditions with increased fibrinolysis and 5) conditions with inhibitors in the blood belong to the plasmatically induced haemorrhagic di atheses

Hereditary Thrombocytogenic Haemorrhagic Diatheses

This group comprises various haemorrhagic diatheses all congenital and the majority still of unknown aetiology

These diseases are 1) Thrombopenia (usually acquired) 2) hereditary haemorrhagic thrombasthenia (Glanzmann) 3) constitutional thrombopathy (Willebrand Jurgens) 4) Naegel s thrombopathy and 5) Jurgens thrombopathy

The clinical pictures of these diseases will not be described in detail

The bleeding tendency in thrombopenia manifests itself by suffusion of blood in the skin and mucous membranes as well as in the brain In the various forms of thrombopathy the haemorrhages are reminiscent of those in haemo philia (hence the name of pseudohaemophilia) They often occur periodically either in early infancy or about the age of puberty

Common to the thrombocytogenic haemorrhagic diatheses are the prolonged clotting time the delayed clot retraction and the reduced conversion of pro thrombin into thrombin

AHF deficiency has been demonstrated in a few instances of von Wille brand Jurgens disease (*Larrieu & Soulier* 1953 *Alexander & Goldstein* 1953 *Jurgens Lehmann Wegelius Erikson & Hiepler 1957 Biggs & Macfarlane* 1957 *Nilsson M Blomback Jorpes B Blomback & Johansson* 1957)

All the diseases of this group are due to dominant genes

The results of the most commonly employed laboratory tests in plasmatic ally induced haemorrhagic diatheses and thrombocytogenic haemorrhagic dia theses are shown in the table fig 3

Vascularly Induced Haemorrhagic Diatheses

This group comprises primarily hereditary *haemorrhagic telangiectasia* (Rendu Osler) which is inherited as a dominant disease and further characterised by repeated haemorrhages from visible telangiectases in the skin and mucous membranes

Vascular pseudohaemophilia is due to a dominant gene occurring like the above diseases in both males and females The haemorrhages in this disease are highly reminiscent of those in classical haemophilia being often intra and periarticular *Macfarlane* (1941 quoted 1953) has observed abnormally long and tortuous capillaries with poor contractility in this disease

Fig 32
Localisation of a clotting defect

Quick s prothrombin time	normal	normal	normal	normal	prolonged	prolonged
clotting time	prolonged or normal	normal or prolonged	normal or prolonged	normal	indefinite	prolonged
bleeding time	normal	prolonged	prolonged	prolonged	normal	normal
capillary resistance	normal	impaired	normal or impaired	impaired	normal	normal
thrombocyte count	normal	<100000	>100000	>100000	normal	normal
diagnosis	HAEMOPHILIA	THROMBOPENIC PURPURA	VON WILLEBRAND S DISEASE	THROMBOASTHENIA GLANZMANN	AFIBRINOGENAEMIA	»HYPOPROTHROMBINAEMIA« HYPOFIBRINOGENAEMIA HEPARINAEMIA INHIBITOR

Finally mention must be made of the idiopathic vascular abnormalities in which conditions apparently unprovoked haemorrhages occur from the pulmonary vessels (pulmonary haemosiderosis) and from the stomach and intestines (idiopathic haematemesis and melaena)

Symptomatology

Detailed descriptions have been given previously of the symptomatology of haemophilia (see *Schloessmann* 1930 and *Andreassen* 1943 among others) Hence only a brief account will be given of it in this work

The most essential signs is the spontaneous as well as postraumatic bleeding tendency Haemorrhages may occur anywhere in the organism but are most often localised within certain areas Skin mucous membranes viscera (stomach intestines kidneys) muscles and joints Spontaneous haemorrhages are rare in the central nervous system (*Skold* 1944 *Schiller Neligan & Budtz Olsen* 1948) possibly owing to the high thromboplastic activity of the brain tissue

Conversely the very frequent and severe bleeding tendency in the joints may be due to the very low thromboplastic activity in the synovial membrane as well as in the fibrous capsule (*Astrup & Sjølin* 1958)

Most haemophiliacs state that their bleeding tendency is periodic Bleeding

at one site is frequently followed by bleeding at various other sites A few writers state that the bleeding tendency is most pronounced in spring and autumn (e g *Virschow* 1854 *Gunder* 1938)

The haemophilic bleeding presents two characteristic features One is that it is a diffuse tissue bleeding and the other is the secondary haemorrhage which may occur several days after a possible primary haemostasis

The statements concerning the relation between the clotting defect and the bleeding tendency differ appreciably

Reports are available on haemophiliacs with a normal clotting time (*Mer skey* 1951 a *Graham McLendon & Brinkhous* 1953) but whose clotting defect was demonstrable by quantitative determination of the AHF content

Some writers especially the fairly old are of the opinion that there is proportionality between the bleeding tendency and the prolongation of the clot ting time (*Weil* 1906 *Addis* 1911 *Schloessmann* 1912) and others that the symptomatology is rather independent of this (*Morawitz* 1930 *Schultz* 1922 *Hecht* 1941–1942 *Quick* 1951)

First Manifestation of the Bleeding Tendency

Andreassen found that 74 out of 128 haemophiliacs presented the first signs of their disease about the age of 12 months In the majority of the remainder the disease had manifested itself before the age of 8 years while only two were over 10 when the first abnormal bleeding occurred Five were manifest bleeders already at birth

In *Neiger's* (1951) series 15 haemophiliacs had the first bleeding within the first year of life 19 within the second year 10 within the second to fifth years 3 within the fifth to seventh years and 1 within the seventh to ninth years In the cases of 55 out of 103 the time of the first bleeding is not specified

Pianta (1933) reports a case in which haemophilia was not recognized till the age of 35

Variations of the Bleeding Phenomena During Life

The bleeding tendency of haemophiliacs is often stated to alter with increasing years The disease seems to become increasingly severe until the age of 20 (*Schloessmann* 1924 *Andreassen* 1943) presumably because this is the most active period of life

After the age of 20–30 a decrease may occur of the signs and symptoms without the clotting defect disappearing A few patients may become almost symptom free Practically complete cessation of bleeding phenomena was also observed in a few of the cases under review where the clotting defect was then no longer demonstrable (see family 52 p 256 and family 56 p 260)

Permanent Changes

The main and most disabling permanent changes caused by the bleeding in haemophiliacs are those of articular deformations in the regressive stage This stage is characterised by contracture subluxation sometimes bony ankylosis and atrophic muscles At this stage of joint damage the haemarthroses apparently cease

Andreassen was informed about the presence of haemarthroses in 135 (81 per cent) out of 166 haemophiliacs Ninetytwo of these had permanent changes in the form of limited mobility and abnormal contours of the joints *Hecht* (1955) states that two-thirds of haemophilic patients present permanently deformed joints

Another but rarer cause of the disablement of haemophiliacs is that of intramuscular haemorrhages which may exert pressure on nerves and thus give rise to persistent neurological changes (e g families 2 13 53 and 111)

Average Longevity of Haemophiliacs

Andreassen on the basis of the ages at death of 105 haemophiliacs found the average longevity to be 18 years

Prognosis

According to *Schloessmann* (1930) about 34 per cent of haemophiliacs bleed to death within the first two decades After that age the lethality decreases appreciably with increasing years

Attention was early called to the fact that the prognosis is better in families presenting the disease in a fairly mild form than in severely affected families *(Andreassen)*

Skold (1944) found the risk of death within the first decade to be 6 8 times greater for haemophiliacs than for normals while within the second and third decades it was 4 9 and 7 5 times greater respectively

Symptomatology in the Different Forms of Haemophilia

Fairly little information is available on the bleeding tendency in the different forms of haemophilia

R L Rosenthal (1954) found that classical haemophilia (AHF deficiency) most often manifested itself by haemorrhages into the joints and the skin as well as by haematuria These manifestations were rare in patients with PTA deficiency The latter patients were primarily liable to haemorrhages following dental extractions and other surgical interventions whereas haemarthroses were rare The bleeding tendency in Christmas patients was found to be intermediate in severity between these two groups

Verstraete (1955) and *Koller* (1955 a) found no symptomatologic difference between classical haemophilia, Christmas disease, and combined haemophilia

Graham McLendon & Brinkhous (1953) have described a mild form of classical haemophilia (antihaemophilic factor deficiency) in which the bleeding tendency manifested itself solely by post traumatic haematomas prolonged bleeding from cuts and after tonsillectomy, as well as after dental extraction

Christmas disease often manifests itself by a severe bleeding tendency with haemarthroses and intramuscular haemorrhages (*Aggeler White Glendening Page Leake & Bates* 1952 *Creveld & Paulsson* 1953)

Biggs & Macfarlane (1957) hold that the Christmas group includes a greater number of mild cases than the group with AHF deficiency since only four out of 11 Christmas patients had severe clinical signs

PTA deficiency is universally agreed to be associated with a small bleeding tendency spontaneous haemorrhages being rare (*R L Rosenthal* 1954 *Ramot Angelopoulus & Singer* 1955 b) *Oehme & Hagette* (1955) described two brothers with PTA deficiency, of whom one had haemarthroses however

The Hageman trait is characterised by absence of manifest haemorrhages despite the most often severe clotting defect (*Ratnoff & Margolius* jr 1956 *Frick & Hagen* 1956 *Ramot Singer Heller & Zimmermann* 1956 *Sjølin* 1957 b) Pronounced abnormal bleeding phenomena (subcutaneous haematomas gingival haemorrhages and haemarthroses) have been observed in only one patient so far with the Hageman trait (*Sjølin* 1957 d)

Summarising we may say on the basis of the literature that the great majority of patients with PTA deficiency have a very slight bleeding tendency and that patients with the Hageman trait generally have no haemorrhagic diathesis whatever As for classical haemophilia and Christmas disease their symptomatology may vary considerably both severe and mild forms being known Clinically these diseases are indistinguishable

Chapter VIII

CLASSIFICATION OF 148 HAEMOPHILIACS BY THROMBIN GENERATION TEST

1 Deficiency of Antihaemophilic Factor (Classical Haemophilia)

A diagnosis of AHF deficiency on the basis of the thrombin generation test is only justifiable if the defective thromboplastic activity becomes normal by adding adsorbed bovine plasma but not by adding normal serum (see the table fig 6 p 46)

It soon appeared however that this group comprising 79 patients could be further divided into subgroups 1) one in which the clotting defect disappeared by merely adding adsorbed bovine plasma (61 patients) 2) One in which the clotting defect disappeared only after addition of adsorbed bovine plasma to patient plasma frozen with its normal content of platelets or by simultaneous addition of adsorbed bovine plasma and heated readsorbed serum (18 patients) This group will be described under combined clotting defects 3) Finally a group was found in which the clotting also became normal after addition of adsorbed bovine plasma but not after addition of normal serum It differs however from the group of ordinary AHF deficiency by the clotting becoming normal after addition of readsorbed normal serum In this group are further included ten patients with slight AHF deficiency (see p 81)

Isolated antihaemophilic factor deficiency was demonstrated in 61 patients (about 40 per cent of the whole series) belonging to the families 1 2 10 13 16 31 34 39 45 54 57 60 64 66 68 69 70 71 72 73 76 78 79 82 84 87 91 94 98 100 103 104 105 107 110 and 111 a total of 36 families

The most important data of these patients are collected in the table fig 33 The patients ages ranged from 6 months to 56 years averaging just under 19 years

Symptomatology

In six patients the first sign of a haemorrhagic diathesis was present at birth In 44 patients the disease manifested itself within the first year of life and in eight within the second year One patient had his first abnormal bleeding at the age of 3 one at 7 and one at 10

Fig

Antihaemophilic factor deficiency

Family	Patient no	Age	First manifest (year)	Milder with er years)	Haemarthroses	Permanent joint deformations	Bleeding in skin	Bleeding in muscles	Gastro intestinal haemorrhages	Urinary tract haemorrhages
1	8	30	3	20	+	—	—	—	—	+
2	11	36	6/12	—	+	+	+	+	—	+
—	12	34	1	—	+	+	+	+	+	+
—	14	25	3/12	—	+	+	+	+	—	—
—	15	22	1	—	+	+	+	+	—	+
—	16	18	1	—	+	+	+	—	—	—
—	17	12	6/12	—	+	+	+	—	—	—
—	18	9	10/12	—	+	—	+	+	—	—
10	2	42	6/12	30	+	+	+	+	—	+
—	3	35	6/12	20	+	+	+	—	—	+
—	4	14	1	—	+	—	+	—	—	+
13	5	56	1	—	+	+	+	+	+	—
—	8	76	4/12	—	+	+	+	+	—	+
16	4	20	1/12	—	+	+	+	+	—	+
31	8	50	10	32	+	+	+	+	+	—
—	9	49	2	19	+	+	+	+	+	—
34	9	11	6/12	—	+	—	+	—	—	—
—	10	12	0	—	+	+	+	—	—	—
39	25	31	1	21	+	—	+	+	+	—
—	24	11	6/12	—	+	—	+	+	—	—
45	8	31	2/12	18	+	+	+	+	—	+
—	9	33	1/12	21	+	+	+	+	+	+
54	2	31	1	—	+	+	+	+	—	+
—	4	22	6/12	—	+	+	+	—	—	+
57	2	43	2	16	+	+	+	+	—	+
60	2	38	1	15	+	—	—	—	—	+
64	5	44	2	+	+	+	+	+	—	+
66	2	17	1	—	+	+	+	—	+	+
—	4	10	1	—	+	+	+	+	—	—
68	1	18	1	—	+	+	+	+	—	+
—	3	16	6/12	13	+	—	+	+	—	+
69	1	6	1	—	+	—	+	—	—	—
70	2	13	1	+	+	+	+	—	—	+
71	3	9	0	—	+	+	+	—	—	—
—	4	7	14/12	—	+	+	+	—	—	—
72	1	11	6/12	—	+	+	+	+	—	—
73	1	5	2/12	—	+	—	+	+	—	—
76	3	4	3/12	—	—	—	+	+	+	—
78	1	13	0	—	+	—	+	—	—	+
—	2	11	3/12	—	+	+	+	—	—	—
—	3	5	3/12	—	—	—	+	—	—	—
79	1	16	18/12	—	+	+	+	+	—	+
—	2	13	2	—	+	+	+	—	—	—
82	1	18	1	—	+	+	+	—	—	+
84	2	27	1	23	+	+	+	+	—	+
—	3	18	4/12	—	+	+	+	—	—	—
—	5	8	1		+	+	+	—	—	—

33

(classical haemophilia)

Gingival haemorrhages	Bleeding of central nervous system	Epis	Lo esp t t	Oth	Fit to work	Occupation	Rec f t me
+	−	−	−	−	fully	technical assistant	12
+	−	+	−	−	partially (D B)	fashion designer	7 40
+	−	+	−	−	partially (D B)	artist	10
+	−	−	−	−	partially (D B)	artist	5
+	−	+	−	−	under care of mental defectives	shoemaker	9 30
−	−	−	−	−		schoolboy	6
−	−	−	−	+		schoolboy	7 40
+	−	−	−	+		schoolboy	10 45
+	−	+	−	−	fully	head clerk	8 22
+	−	−	−	−	fully	mechanic	6 50
−	−	−	−	−		schoolboy	7
+	−	+	−	−	partially	farm owner	16
+	+	−	−	−	*fully*	*LL B*	*15*
+	−	+	−	−	partially	watch maker s apprentice	8 30
+	−	−	−	−	fully	carrier	7 [illegible]0
+	−	−	−	−	fully	tailor	7 45
+	−	−	−	−			11
−	−	−	−	+			19
+	−	−	−	−	fully	provision dealer	9
+	−	−	−	−			12 30
+	−	−	−	−	−(D B)	IR	12 30
+	−	+	−	−	−(D B)	accountant	9 16
+	−	+	−	−	−(D B)		11 30
+	−	+	−	−	−		9
+	−	+	−	−	fully	business man	7 45
+	−	+	−	+	fully	watch maker	1[illegible] 45
−	−	+	−	−	partially	rentier	12 10
+	−	+	−	−			14
−	−	−	−	−			18 15
−	−	+	−	−	−		14
−	−	−	−	−	+	optician s apprentice	15
−	−	+	−	+			4 30
−	−	+	−	+		schoolboy	7
+	−	−	−	−		schoolboy	10
−	−	−	−	−		schoolboy	8 9
−	−	−	−	−			12
−	−	−	−	+			15
−	−	−	−	+			10 30'
−	−	−	−	−		schollboy	5 22
+	−	−	−	−		schoolboy	4 45
−	−	−	−	+			5 30
−	−	−	−	+	−		13 [illegible]0
+	−	−	−	−	−		8 15
−	−	+	−	−	−	D B	11 30
+	−	+	−	−	−	D B	1[illegible] 30
+	−	−	−	−	−		9 48
+	−	+	−	−			14

Fig 33

Family	Patient no	Age	First manifest (year)	Milder with (Iner years)	Haemarthroses	Permanent joint deformation	Bleeding in skin	Bleeding in muscles	Gastro-intestinal haemorrhages	Urinary tract haemorrhages
—	4	9	1	—	+	—	+	—	—	—
87	1	7	7	—	—	—	—	+	—	—
91	1	7	18/12	—	+	—	+	—	—	+
94	1	15	18/12	13	+	—	+	—	—	—
98	5	18	6/12	—	+	—	+	—	—	—
100	1	2	0		—	—	+	—	—	—
103	1	7	0	—	+	—	+	+	—	+
104	1	15	6/12	+	+	+	+	—	—	+
105	1	3	0	—	+	—	—	—	—	—
—	2	6/12	5/12		—	—	—	—	—	—
107	1	16	1	—	+	—	+	—	—	—
110	1	4	3/12	—	+	—	+	—	—	—
111	1	18	1	—	+	+	+	—	—	—
—	2	2	1		+	—	+	—	—	—
36 families	61 patients				56+	36+	56+	27+	7+	27+

) + indicates that the disease had grown milder in the course of years A figure in the column indicates the patient's age at years at which the disease began to abate
D B = disablement benefit

56 of the 61 patients of this group had had haemarthroses, in 36 cases resulting in permanent changes 26 out of 33 patients over 15 years of age presented permanent joint deformities Fifty six of the patients had had haemorrhages into the skin

These two forms of haemorrhage were by far the most frequent in this group Next in frequency followed gingival haemorrhage which had been experienced by 35 i e just over half of the patients 27 had had intramuscular haemorrhages 27 haematuria 26 epistaxis 7 gastro intestinal haemorrhage and 16 bleedings elsewhere e g in an eye from the lobule of the ear after blood sampling from the frenulum of the upper lip from the lips and tongue etc

Haemorrhage in the central nervous system had occurred in one patient only after a severe injury (family 13 no 8) None had bled from the lower respiratory tract

15 patients about 25 per cent pronounced that the disease had grown milder in the course of years The alleged age of beginning abatement varied somewhat from 13 to 32 years

11 patients or barely one fifth were fit in the sense that they received no disablement benefit even though they were troubled periodically by haemorrhages and a few were fairly disabled

(continued)

Gingi l h emo-rrh ges	Bleeding in cent l nerv s ystem	Epistaxis	Lower resp tract	Other	Fit to work	Occup on	Recalc fication-time
–	–	–	–	–			9 45
+	–	–	–	–	–		19
–	–	+	–	–	–		7 40′
+	–	+	–	–	+	painter s apprentice	7 45
–	–	+	–	–	partially		7 45
–	–	–	–	+			12
+	–	+	–	–	–	–	7 30
+	–	+	–	+			9 50
–	–	–	–	+			11 45
–	–	–	–	×			16
+	–	+	–	–			7
–	–	–	–	–			6 30
+	–	–	–	–	partially	reads for the General Certificate	7 30
–	–	–	–	+			12 30
35+	1+	26+	0	16+			

27 of the total series were 18 years old or more Nine of these were able to support themselves nine were partially fit of whom two were students

Nine of the adults were totally disabled in the sense that they were unable to perform manual work Eight patients received disablement benefit Four of these were able to add to their income by doing sedentary work None of the patients of this group had done their military service

The Clotting Defect

The recalcification times in diluted plasma from these patients ranged between 4 min 45 sec and 16 min Twenty seven were over 10 min The defective thrombin generation was normalised by adsorbed bovine plasma but not by serum though addition of serum most often gave a considerable fall of the recalcifi cation time

In most cases within this group there was only negligable thrombin genera tion within the 13 minutes of observation Samples from no more than seven patients formed appreciable amounts of thrombin and only after a greatly prolonged lag period (family 2 nos 11 and 16 family 10 nos 2 and 3 family 31 no 8 family 70 no 2 and family 104 no 1) In another three (family 10 no 4 family 69 no 1 and family 107 no 1) some though con

siderably smaller amounts of thrombin were formed One of these (family 107 no 1) obtained a normal thrombin generation after the plasma had been stored with its normal content of platelets at –20° C

Among the plasma samples in which no appreciable thrombin formation took place, eight were tested for thrombin generation after the plasma had been stored at –20° C with the normal content of platelets This gave a normal thrombin generation in only one sample (family 57 no 2)

In nine samples the effect of adding platelet suspension was investigated *In two (family 10 nos 2 and 4) the thrombin formation improved so much* that it must be characterised as normal Both patients belonged to the group with some though delayed thrombin generation The recalcification time became normal in seven of these nine samples

Fresh normal plasma was added in four cases This gave normal thrombin generation and recalcification time in all four samples

Discussion

In all the cases belonging to the group of AHF deficiency the thromboplastic defect was easy to detect by means of the thrombin generation test even in the few instances where the recalcification time was normal or very nearly so *It is remarkable that in these cases (e g family 78) the thrombin generation* test showed no appreciable thrombin generation not even after a prolonged lag period It is likewise remarkable that even grave clotting defects may be associated with almost complete freedom from haemorrhagic manifestations (e g family 60 no 2)

Conversely cases were found of very severe haemophilia causing pronounced disablement in which the recalcifaction time was only moderately prolonged and in which considerable amounts of thrombin were formed (family 2 no 11)

Patients with an appreciable but delayed thrombin generation may possibly have only a mild degree of antihaemophilic factor deficiency If so it is strange that some of these patients are more ill than patients with a poorer *thromboplastic activity in the plasma This suggests that other pathogenic fac*tors exist besides the AHF deficiency and that these do not manifest themselves in the investigation of the coagulation system

While the clinical picture in the group with AHF deficiency is a very *heterogeneous one the thrombin generation is in most cases uniformly poor* A somewhat greater variation is seen in the recalcification time

In the series under review the cases of pure AHF deficiency constitute no more than about 40 per cent of the total number of patients *This is a very small proportion* compared with those stated by other investigators (see the table fig 51) but is in part accountable for by the subdivision of the AHF

group If we include the 18 patients who lack the AHF plus freezing/serum factor the group constitutes about 50 per cent which however is still a very small proportion of the total series This problem will be discussed later

Classical Haemophilia and Readsorbed Serum

The group of AHF deficiency comprises in addition to the main group a small one which is of particular interest from a coagulative point of view As in the remaining AHF patients the clotting defect disappeared after addition of adsorbed bovine plasma but not after addition of normal serum This group is however distinguished by the clotting defect being also eliminated after addition of the same normal serum adsorbed twice with 20 mg barium sulphate per millilitre of serum (*Sjølin* 1957)

This phenomenon was demonstrated in eight out of 61 patients investigated (e g family 71 no 4) In another six the thrombin generation improved somewhat without becoming quite normal The recalcification time was shortened in all 61 patients except four No explanation can at present be given of this phenomenon but the possibility must be considered that an inhibitor is inactivated by the adsorbed serum Another possibility is that AHF is present in serum in an inactive form linked to an inhibitor (*Bergsagel & Biggs* 1955) It might be conceived to be liberated from its bound state by adsorption This small group differs neither clinically nor genetically from the remaining patients with AHF deficiency

2 Slight Antihaemophilic Factor Deficiency

Introduction

In continuation of the account of the patients with classical haemophilia there is reason to describe a group of patients the majority with mild haemophilic signs and symptoms in whom the thromboplastic defect is probably due to a mild degree of antihaemophilic factor deficiency

At the beginning of the investigation these patients were classified as lacking either the Rosenthal factor (plasma thromboplastin antecedent) or the Hageman factor because the clotting defect of blood samples from these patients was normalised by adsorbed bovine plasma as well as by normal serum and heated readsorbed normal serum respectively However these two groups of PTA deficiency and Hageman factor deficiency became so large comprising 13 and 18 patients respectively that inclusion of other presumably mild defects were suspected The suspicion was confirmed by cross tests between blood samples from these groups and samples with known pronounced deficiency of the AHF or of the Christmas factor

Of the patients classified primarily under the heading of PTA deficiency eight were found by cross tests to have AHF deficiency (families 31, 67 88 99 101) two deficiency of the Christmas factor (family 23) and two combined defect (family 41 see p 109) Among the patients classified primarily under the heading of Hageman trait three proved to have AHF deficiency (families 18 88 and 92) and four to lack the Christmas factor (families 8 and 23) (see p 91)

The group of *slight AHF deficiency* comprises 11 patients The most important data are shown in the table fig 34 The patients ages ranged between 9 and 81 averaging 33 years Five were over 25 years of age

Symptomatology

Two patients displayed signs of a haemorrhagic diathesis from birth The average age at the first manifestation of the disease was about 4 years Eight had manifest signs before the age of 3 One stated that the disease was first noticed in relation to an operation at the age of 39 This information given by an 81 year old patient should probably be accepted with some reservation Yet he had been a blacksmith and had taken part in the Boer War as a private so his disease is unlikely to have caused him any great trouble

All eleven patients had had bleedings into the skin while nine had bled

Fig

Slight AHF

Family	Patient no	Age	First manifest (years)	Mild with[illegible]nce years	Haemarthroses	Permanent joint deformations	Bleeding in skin	Bleeding in muscles	Gastro-intestinal haemorrhages	Urinary tract haemorrhages
18	8	27	2	–	+	+	+	+	–	+
31	6	49	2	39	+	+	+	+	–	+
67	1	11	0	+	+	–	+	–	–	–
88	2	81	39	?	–	–	+	–	+	–
–	3	21	2	14	–	–	+	–	–	–
–	4	19	11/12	–	+	–	+	–	–	–
92	2	37	1	?	–	–	+	–	–	–
99	2	9	0	–	–	–	+	–	–	–
101	2	67	?	–	+	+	+	–	–	–
–	4	22	1	+	+	–	+	–	–	–
–	5	19	6/12	+	+	–	+	–	–	–
7 families	11 patients			5	7+	3+	11+	2+	1+	?+

Average age 33 years
First manifestation at 3–4 years of age

from the gums and seven had had haemarthroses Three adults presented permanent joint deformities and three did not

Five patients had been troubled by episodes of protracted epistaxis one by gastro-intestinal haemorrhages two by haematuria two by intramuscular haemorrhages and two by other haemorrhages

Five declared that the disease had grown milder in the course of years

The nine adults were fully fit two of these a master carpenter and a goldsmith in spite of pronounced permanent joint deformities Five had jobs requiring great physical effort a blackmith unskilled labourers a grocer and a carpenter *while only four had more sedentary occupations* a *tailor* a *gold* smith and students

The Clotting Defect

The recalcification times ranged between 4 min and 7 min 25 sec Five had recalcification times under 5 min

The thrombin generation test showed a considerable though most often greatly delayed thrombin generation in blood from all these patients (e g family 31 no 6) In two the thrombin generation was perfectly normal (fam ily 88 nos 3 and 4)

Fig 35 shows the results of the most important experiments with thrombin

34

deficiency

G g l h mo- rrh ges	Bl d g ce al er us ystem	Epist is	Low esp t act	Oth	F t to w k	Occup t	Rec l fi t t m
+	–	+	–	–	partially	Goldsmith	7
+	–	–	–	+	fully	Master tailor	6 50
+	–	–	–	–			6 15
+	–	+	–	–	fully	former blacksmith	4 15
–	–	+	–	+	fully	unskilled labourer	4 20
–	–	+	–	–	fully	unskilled labourer	4
+	–	–	–	–	fully	Grocer	5 40
+	–	–	–	–			5 30
+	–	–	–	–	partially	Master carpenter	4 15
+	–	+	–	–	fully	Training college student	7 25
+	–	–	–	–	fully	Training college student	4 5
9+	0	5+	0	2+	9	Average	5 25

generation test as well as the recalcification times in plasma samples from this group of patients

The clotting defect was in all cases corrected by adsorbed bovine plasma and by serum In four out of ten the defect was corrected by heated readsorbed serum Strangely enough the thrombin formation and the recalcification time deteriorated in blood from the two patients with a normal thrombin generation (family 88 nos 3 and 4) after addition of heated readsorbed serum

In six out of seven plasma samples the thrombin generation became normal after addition of a platelet suspension stored at −20° C In samples from four patients fresh platelet suspensions gave normal thrombin generation in all four cases In one of these the thrombin generation had not been normalised by a frozen platelet suspension

Eleven samples were tested after having been stored at −20° C with their normal content of platelets In seven cases the thrombin generation became normal

In two instances the results of freezing of platelet rich plasma and those of addition of frozen platelet suspension were inconsistent

In five cases the effect of freezing on platelet poor plasma was investigated The number of platelets varied between 1000 and 7000 per μl of plasma In three samples the thrombin generation became normal Platelet poor and plate let rich plasma reacted equally to freezing

Finally in all out of seven samples investigated readsorbed serum nor malised the thrombin generation

All the plasma samples were submitted to cross tests with plasma lacking the AHF and with plasma lacking the Christmas factor All the stated plasma samples normalised the thrombin generation in plasma lacking the Christmas factor but not that in plasma lacking the AHF

Discussion

The form of haemophilia described above is milder both clinically and haema tologically than the classical AHF deficiency

The first sign of a bleeding tendency occurred somewhat later (at the age of 4 years) than the first manifestation of the classical AHF deficiency (at the age of 1 year)

The average age was somewhat higher 33 against 19 One third of the adults (three out of nine) had developed permanent joint deformities against four fifths of the adults with classical haemophilia

A scant half declared that the disease had grown milder in the course of years The corresponding figure for classical haemophilia was about one fourth

All the adults of this group were fit the majority even able to do fairly strenuous physical work For patients with classical haemophilia the correspond ing figure lay between one half and one third

Fig 35

Slight AHF deficiency

I b t m t							F o pl t l t ich plasma	F pl l t poor plasm)			
ml of patient plasma		1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0
ml of adsorbed bovine plasma			0 2								
ml of serum				0 2							
ml of readsorbed serum									0 2		
ml of heated readsorbed					0 2						
ml of 0 85 NaCl		1 0	0 6	0 8	0 8	0 8	1 0	1 0	0 8	0 6	0 8
ml of CaCl		1 0	1 2	1 0	1 0	1 0	1 0	1 0	1 0	1 2	1 0
frozen platelet suspension						0 2					
normal plasma										0 2	
fresh platelet suspension											0 2
Family	*Patient no*										
18	8	delayed 7	normal 3 15	normal 3 30	normal 4 10		poor 2 30		normal 4		
31	6	delayed 6 50	normal 3 30	normal 3 33	delayed 6	normal 4 45	poor 5 30	poor 6 .5			
67	1	delayed 5 15	normal 2 39	normal 2 27	delayed 6 15	poor 6 30	poor 4 30	poor 9 50	normal 4 50		
88	2	delayed 4 15	normal 4 35	normal 2 45	normal 3 35	poor 4 40	normal 3 0	normal 3 30			normal 2 40
–	3	normal 4 .0	normal 3 30	normal 2 .0	poor 6 10	normal 3 25	normal 4		normal 2 10		normal 3 16
–	4	normal 4	normal 3 .0	normal 2 10	normal 4 30	normal 4 55	normal 3 50		normal .30		normal 2 27
92	2	delayed 5 40	improved 3 40	normal 3 70	normal 3 50	normal 3	normal 4	normal 2 35			
99	2	delayed 5 30	normal 2 30	normal 3 15	poor 6	normal 4 30	normal 1 15	normal 1 58			
101	2	delayed 4 15	normal 3	normal 3 15	delayed 4 10	normal 4 30	normal 5 6		normal 3 .0		normal 3 50
–	4	delayed 7 25	normal 3 70	normal 1 45	poor 2 50		normal 4 45		normal 2 15		
–	5	delayed 4 5	normal 4	normal 4			delayed 4 30		normal 3	normal .50	

) Th mbe f plat l ts (th t t d pl ma ss ples w 1000 4000 7000 5000 7000 r pecti ly

generation test, as well as the recalcification times in plasma samples from this group of patients

The clotting defect was in all cases corrected by adsorbed bovine plasma and by serum In four out of ten the defect was corrected by heated readsorbed serum Strangely enough the thrombin formation and the recalcification time deteriorated in blood from the two patients with a normal thrombin generation (family 88 nos 3 and 4) after addition of heated readsorbed serum

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Fig 35

Slight AHF deficiency

I b t m t							F ozen pl t l t i h plasm	F pl t po plasm)			
ml of patient plasma		1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0
ml of adsorbed bovine plasma			0 2								
ml of serum				0 2							
ml of readsorbed serum									0 2		
ml of heated readsorbed					0 2						
ml of 0 85 / NaCl		1 0	0 6	0 8	0 8	0 8	1 0	1 0	0 8	0 6	0 8
ml of $CaCl_2$		1 0	1 2	1 0	1 0	1 0	1 0	1 0	1 0	1 2	1 0
frozen platelet suspension						0 2					
normal plasma										0 2	
fresh platelet suspension											0 2
Family	*Patient no*										
18	8	delayed 7	normal 3 15	normal 3 30	normal 4 10		poor 2 30		normal 4		
31	6	delayed 6 50	normal 3 30	normal 3 33	delayed 6	normal 4 45	poor 5 30	poor 6 25			
67	1	delayed 5 15	normal 2 39	normal 2 27	delayed 6 15	poor 6 30	poor 4 30	poor 9 50	normal 4 50		
88	?	delayed 4 15	normal 4 35	normal 2 45	normal 3 35	poor 4 40	normal 3 _0	normal 3 30			normal _ 40
–	3	normal 4 _0	normal 3 30	normal 2 0	poor 6 10	normal 3 25	normal 4		normal 2 10		normal 3 16
–	4	normal 4	normal 3 0	normal 2 10	normal 4 30	normal 4 55	normal 3 50		normal 2 30		normal 2 ?7
9?	2	delayed 5 40	improved 3 40	normal 3 _0	normal 3 50	normal 3	normal 4	normal 2 35			
99	2	delayed 5 30	normal 2 30	normal 3 15	poor 6	normal 4 30	normal 1 15	normal 1 58			
101	2	delayed 4 15	normal 3	normal 3 15	delayed 4 10	normal 4 30	normal 5 76		normal 3 20		normal 3 50
–	4	delayed 7 25	normal 3 70	normal 1 45	poor 2 50		normal 4 45		normal 2 15		
–	5	delayed 4 5	normal 4	normal 4			delayed 4 30		normal 3	normal 2 50	

) Th mb rs of pl t l ts th t t d pl m mples w e 1000 4000 7000 5000 7000 espect ly

The cases running a fairly mild clinical course presented a correspondingly less pronounced clotting defect In two the thrombin generation test revealed no defect whatever It is also remarkable that serum whether untreated, ad sorbed or adsorbed plus heated contains a factor that can replace the AHF Theoretically it may be a question of very small amounts of AHF This is however fairly unlikely, because AHF is supposed to be inactivated during the clotting Moreover, it is destroyed by heating It is more reasonable to suppose that other factors which in part make up for the action of the AHF are formed in plasma during the clotting This might explain why serum added to plasma from patients with classical haemophilia always brings about a con siderable reduction of the recalcification time

The results of the platelet experiments and the freezing experiments might perhaps bear out this hypothesis as these also most often showed a fall in the recalcification time and normalisation of the thrombin generation The disintegration of the platelets possibly causes liberation of a factor which can substitute the AHF and is recovered in serum In the experiments with addition of normal platelets there was, however a possibility that these carried AHF adsorbed on the surface

It is worth mentioning in this place that *M C Rosenthal* (1954) found that in some instances addition of serum to plasma lacking the AHF may result in a normal clotting time and recalcification time but not in normal prothrombin consumption

The two other haemophiliacs belonging to family 31 were not classified in this group despite the mild manifestation of the disease and the rather appreciable thrombin formation because addition of serum did not normalise the thromboplastic activity of their plasma as was characteristic of the stated group so that subsequently there was no diagnostic difficulty

3 Christmas Factor Deficiency (plasma thromboplastin component)

A diagnosis of Christmas disease was made in the cases of haemophilia where the thromboplastic defect in the plasma was corrected by normal serum but not by adsorbed bovine plasma nor by heated readsorbed serum

Christmas factor deficiency was demonstrated in 27 males belonging to the families 4 8 14 22 23 36 43 51 53 62 83 85 86 93 97 and 102

In three of these the differential diagnosis against the Hageman trait was difficult (family 36 nos 2 and 3 and family 83 no 1) In two of these case addition of heated readsorbed serum greatly improved the thromboplastic ac tivity (family 36 nos 2 and 3) whereas it did not shorten the recalcification time and the lag period in the thrombin generation test so much as is usually seen in patients lacking the Hageman factor Further adsorbed bovine plasma was unable to compensate the clotting defect as it does in the cases of the

Hageman trait Finally the thrombin generation curves showed practically no thrombin formation in contrast to Hageman plasma which displays considerable, though delayed thrombin generation

In the third patient (family 83 no 1) the thromboplastic activity was hardly reduced though the patient doubtless had a haemorrhagic diathesis Unlike normal serum adsorbed bovine plasma and heated readsorbed serum did not accelerate the thrombin generation

For the stated reasons these three patients have been referred to the Christmas group

Symptomatology

The most important clinical data have been tabulated in fig 36 The patients ages range from 2 to 66 years averaging about 27 Twenty were over 15 years of age

The first sign of a haemorrhagic diathesis had occurred before the age of 16 on an average about the age of 2 years Fifteen had the first abnormal bleeding within the first year of life seven within the second year one at the age of 4 two at the age of 5 one at the age of 13 and one at the age of 16

All the patients had had bleedings in the skin Second in frequency were haemarthroses (21 patients) Fifteen of these all adults had developed permanent joint deformities with limited mobility and arthrotic changes Only two adults (family 8 nos 6 and 8) did not present permanent changes As the third in frequency gingival haemorrhages (18 patients) followed in relation to primary and secondary dentition or after dental extraction Nearly equally frequent (17 patients) were prolonged episodes of epistaxis Eleven had had haematuria 11 intramuscular haemorrhages 5 haemorrhages in the alimentary tract and 3 in the upper respiratory tract or the pleural cavity

Five adults were totally disabled chiefly owing to their joint deformities Another two had such a greatly reduced working capacity as to receive disablement benefit Three adolescents were already now so unfit to work that they will probably have difficulty in learning a trade Altogether one third of these patients had such a reduced working capacity that they must be characterised as invalids

Eight were able to support themselves two of these in spite of a certain degree of disablement

Two patients had done their military service One had had to give up however after 10½ months Seven of the patients declared that the disease had grown milder in the course of years

Fig

Christmas factor

Fam ly	Pat ent no	Age	F rst man fest (y r)	M ld r with in r years	Ha m r throses	P r man t joint deforma t ons	Bleeding in sk n	Bleed ng n m scl	Gast o test n l haemo-r hages	Un ry t act h emo-rrh ges
4	3	66	1	–	+	+	+	+	–	+
–	10	10	2/12	–	+	–	+	–	–	–
8	6	45	5	38	+	–	+	+	–	+
–	8	29	1		+	–	+	–	–	–
14	10	18	6/12	–	+	+	+	–	+	+
22	14	40	1	34	+	+	+	–	–	+
–	15	31	18/12	+	–	+	+	–	–	–
–	17	23	2	–	+	+	+	+	–	–
–	18	16	3/12	–	+	–	+			
–	19	15	1	–	+	+	+	–	–	+
23	3	61	13	+	+	+	+	+	–	–
–	4	60	16	36	+	+	+	+	–	+
–	13	3	2		–	–	+	–	–	–
36	2	43	4	+	+	+	+	+	–	+
–	3	36	1	–	+	+	+	+	+	–
43	3	20	6/12	–	+	+	+	+	+	+
51	3	37	1	+	+	–	+	–	–	–
53	3	22	4/12	–	+	+	+	+	+	+
62	3	17	6/12	–	+	+	+	–	–	+
83	1	51	2	–	–	–	+	–	–	–
–	2	2	0	–	–	–	+	+	–	–
85	1	10	18/12	–	+	–	+	–	–	–
86	1	31	2	–	–	–	+	–	–	–
93	1	10	18/12	–	–	–	+	–	–	–
–	2	8	5		–	–	+	–	–	–
97	2	6	10/12		+	+	+	+	+	–
102	1	18	6/12		+	+	+	–	–	+
16 families	27 patients	17 over 18 years		7+	21+	15+	27+	11+	5+	11+

The Clotting Defect

In these patients the recalcification times ranged between 4 and 20 minutes

Hardly any thrombin generation was seen in plasma from 12 of the patients lacking the Christmas factor The corresponding recalcification times were very long In plasma from 15 patients measurable amounts of thrombin were formed but only after a prolonged lag period The corresponding recalcification times were somewhat shorter than in the preceding group but no more than two were normal (under 5 min) These 15 patients are unlikely to have totally lacked the Christmas factor In one patient the thrombin generation was practically normal as was also the recalcification time (4 min)

36

deficiency

Gingi al haemo-rrh ges	Bleed g i ce t al nerv us sy tem	Epist s	Lower esp t act	Othe	F t to wo k	Occup tio	Rec lc ficatio t m
+	−	+	+	−	− (D B)	−	12
−	−	+	−	−			19 54
+	−	+	−	−	fully	lawyer	7 30
−	−	+	−	−	fully	farmer (soldier)	4 15
+	−	−	−	−	partially	reads for the General Certificate	22
+	−	−	−	−	fully	tailor	10 45
+	−	+	+	−	partially (D B)	tailor	11 56
+	−	−	−	−	partially (D B)	tailor	11 30
							11 30
+	−	+	−	−	partially	watchmaker s apprentice	10 45
+	−	−	−	−	fully	furnace man	7 30
+	−	+	+	−	fully	grocer (soldier)	4
−	−	−	−	+			5 15
+	−	+	−	−	− (D B)		9 34
+	−	+	−	+	− (D B)		11 30
+	−	+	−	−	− (D B)		18
+	−	+	−	−	fully	mechanic	5 50
+	−	+	−	−	−		19
−	−	+	−	−	−		17
+	−	+	−	−	fully	unskilled labourer	4
−	−	−	−	−			7
+	−	−	−	+			8 ₋5
+	−	−	−	+	fully	farm owner	6 45
+	−	−	−	+			7 50
−	−	+	−	+			7 15
−	−	+	−	−			18 35
−	−	+	−	−	partially	business man	6 35
18+	0	17+	3+	7+	8		

The presence of an appreciable though delayed thromboplastic activity presumably indicates that the Christmas factor is not completely absent In one case (see family 8 no 6) it was noticed that at a time when the thrombin generation was normal the patient s plasma could not correct the clotting defect *in plasma from a patient with Christmas disease* when added in the usual amount (0 2 ml) whereas it did so when added in double quantity

Thrombin Generation after Storage of the Plasma at −20° C

In 21 cases the thrombin generation was investigated in plasma stored at −20° C with its normal content of platelets In five the thrombin generation became

normal Two of these are referable to the group with very little or no thromboplastic activity before the freezing while three had had some though delayed thromboplastic activity The remaining 16 including cases with a delayed thromboplastic activity, presented no essential improvement though the re calcification times decreased

In 14 cases the thrombin generation was investigated in vigorously cen trifuged plasma after this had been stored at −20° C In none of these did the thrombin generation become normal

Addition of Platelet Suspension

The effect of adding a normal platelet suspension was studied in 14 cases In two the thrombin generation became normal one severe and one mild In the remaining 12 no change occurred

Discussion

Relatively few works are available dealing with the classification of haemo philic patients *Beaumont Caen & Bernard* (1954) found five Christmas pa tients among 35 haemophiliacs i e about 15 per cent and *Frick* (1954) six Christmas patients among 55 i e about 11 per cent *R L Rosenthal* (1954) found Christmas factor deficiency in six out of 31 patients (about 20 per cent) *M C Rosenthal* (1954) in six out of 40 (12 per cent) and *Soulier & Larrieu* (1953) in four out of 33 (12 per cent) *Stefanini* (1954) estimated that one fifth of all haemophiliacs lack the Christmas factor *Verstraete & Vanden broucke* (1955) found four Christmas patients among 43 patients (9 per cent) and *Fantl & Sawers* (1956) six Christmas patients among 43 haemophiliacs (14 per cent) *Biggs & Macfarlane* (1957) classified 190 patients with haemo rrhagic diathesis 13 had von Willebrand s disease 2 circulating anticoagulants while 13 were not identified 20 had Christmas disease Calculated from the whole series after deduction of the cases of von Willebrand s disease the unidentified and the two with inhibitors there were about 12 per cent with Christmas disease

In the present series 27 out of 148 haemophiliacs i e about 18 per cent had Christmas disease (see however p 91) This corresponds approximately to *R L Rosenthal* s (1954) finding The higher incidence of Christmas disease in the series under review than in most other series described is probably due to the fact that mild cases are easier to trace in a small well defined popula tion A few of these patients have never consulted a doctor about their haemo rrhagic diathesis which was only disclosed by systematic questioning of the members of the family or detected by bleeding after dental extraction for instance Another possibility is that the thrombin generation test is more sen sitive than the prothrombin consumption test and the thromboplastin genera

tion test The two latter methods can both give normal results in mild cases of haemophilia (*Biggs & Macfarlane* 1957)

The author has relatively little experience with regard to these methods but found normal values on thromboplastin generation test in a single Christ mas patient (family 23 no 4) The whole problem ought however to be submitted to more comprehensive investigations using different parallel meth ods of testing the same patient plasma

Among the Christmas patients there was a fair correlation between the ability to generate thrombin the recalcification time and the degree of the clinical manifestations Thus the most severely affected patients had the poorest thromboplastic activity A similar correlation was not always seen in classical haemophilia

Clinically the severe cases of Christmas disease are indistinguishable from classical haemophilia while the mildest cases are difficult to distinguish from the Hageman trait As stated previously the disablement in Christmas disease is considerable About one third of the patients had a greatly reduced working capacity

The clotting defect was corrected by normal plasma and serum In a few instances it was also corrected by the patient s own serum (e g in family 8 and family 86) These were patients whose plasma had a considerable thrombo-plastic activity suggesting that there was not complete absence of the Christmas factor However unlike in the following group normalisation was not obtained with adsorbed bovine plasma

In few cases the clotting defect was compensated by freezing or after addition of normal platelet suspensions The two latter problems ought to be investigated more closely

4 Mild Degree of Christmas Factor Deficiency

This group comprises six patients (see fig 37) The preliminary investigation classified the first four of these as Hageman defects and the last two under the heading of PTA deficiency

The patients ages ranged between 6 and 50 years averaging 23 Two were over 40 years of age

Symptomatology

Only one patient (family 23 no 9) had bled in nearly all the regions where haemophilic haemorrhages usually occur The remainder had had bleedings in few areas only most often in the skin from dental alveoli and the gums In the latter regions the bleedings were frequently so pronounced as to require treatment

Six had had bleedings into the skin five from the gums and three haemar throses One of these three was adult and had developed permanent joint

Fig

Mild Christmas

Family	Patient no	Age	First manifest (year)	Milder within years	Haemarthroses	Permanent joint deformations	Bleeding in skin	Bleeding in muscles	Gastrointestinal haemorrhage	Urinary tract haemorrhages
8	4	50	5	30	–	–	+	+	–	+
–	5	43	5	30	–	–	–	–	–	–
23	9	21	5/12	?	+	+	+	–	+	+
–	10	10	1	?	–	–	+	–	–	–
–	11	6	7/12	?	+	–	+	–	–	–
–	12	6	1/12	?	+	–	+	–	–	–
2 families	6 patients				3+	1+	5+	1+	1+	2+

deformities Two had had haematuria Intramuscular gastro intestinal, and other haemorrhages (frenulum of the upper lip tongue) had occurred in one patient each None had bled in the central nervous system or the lower re spiratory tract

The two oldest patients of the group (brothers) stated that the disease had grown milder since the age of 30 All three adults of this group were able to support themselves and a family Two were farm owners and the third a clerk

The Clotting Defect

The recalcification time was only moderately prolonged In one case it was normal The times ranged between 4 min 45 sec and 6 min 50 sec

All the plasma samples presented considerable thrombin generation but *only after a prolonged lag period*

The thrombin generation became normal in all six cases after addition of adsorbed bovine plasma (The table fig 38 is a survey of the most important experiments performed with this group) The thrombin generation and recalci fication time became normal in all the cases by adding serum in four by *adding heated readsorbed serum*

In all six cases the thrombin generation was tested in plasma stored at –20° C with its normal content of platelets The thrombin generation and the recalcification time became *normal throughout In* two cases platelet poor plasma (19 000 and 6 000 platelets per μl of plasma) was submitted to similar tests *Here* too normalisation was obtained of the thrombin generation and the recalcification time

Addition of fresh platelet suspension normalised the thrombin generation and the recalcification time in three cases In three out of four addition of frozen platelet suspensions gave similar results

37

factor deficiency

Gingi l haemorrh ges	Bleed g i cent l ervous ystem	Epista	Low r resp t act	Oth	F t to work	Occ p t	Rec lc ficatio t me
+	−	+	−	−	fully	farmer	6 45
+	−	+	−	−	fully	farmer	6 15
+	−	+	−	−	fully	clerc	5 30
+	−	−	−	−			4 45
−	−	−	−	+			6 50
−	−	−	−	−			6
4+	0	3+	0	1+	3		

The effect of the patient s own serum on the thrombin generation was investigated in two cases The thrombin generation became normal in both

Finally the effect was investigated of adding the patient s own washed platelets to the plasma from which the platelet suspensions had been prepared This also gave a normal thrombin generation

In supplementary but very important experiments the effects of this group of plasma samples were tested on plasma samples with known Christmas factor deficiency and with known AHF deficiency The thrombin generation and the recalcification time became normal in the plasma samples with AHF deficiency but not in those lacking the Christmas factor

Discussion

The stated group of patients offers points of resemblance to as well as deviations from the Christmas group described above

The average for the two groups was 23 and 27 years respectively In both groups the first manifestation of a haemorrhagic diathesis occurred at the age of 2 or 3 years The mild Christmas defect seems to have manifested itself at a somewhat earlier age than the mild AHF deficiency (see later)

Permanent joint deformities on the other hand seem to be a much rarer phenomenon in this group than in the proper Christmas group All the adult patients were fit The children were not affected or troubled by their moderate bleeding tendency This accords well with the less pronounced clotting defect in this group

The thrombin generation test was in all cases able to disclose the presence of a clotting defect whereas it was impossible to classify this defect in relation to the commonly used normal reagents

This is possibly due to the fact that the Christmas factor is not removed

Fig 38

Mild Christmas factor deficiency

In vb t on m sure					Fro n pl t l t ich pl ma	Frozen platel t poor plasma						
ml of patient plasma	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0
ml of adsorbed bovine plasma		0 2										
ml of serum			0 2									
ml of readsorbed serum											0 2	
ml of heated readsorbed serum				0 2								
ml of 0 85 / NaCl	1 0	0 6	0 8	0 8	1 0	1 0	0 8	0 8	0 6	0 8	0 8	
ml of CaCl	1 0	1 2	1 0	1 0	1 0	1 0	1 0	1 0	1 2	1 0	1 0	1 0
ml of frozen platelet suspension												
ml of fresh platelet suspension								0 2				
ml of plasma							0 2					
ml of own serum									0 2			
ml of own washed platelets										0 2		1 0

Family	Patient no												
8	4	delayed 6 45	normal 2 30	normal 1 45	normal 1 45	normal 4 45		normal 1 30	normal 4 15	normal 4 35	normal 1 50		
8	5	delayed 6 15	normal 3 30	normal 2 30	normal 3 15	normal 2 45	normal 3 40	normal 1 50	delayed 4 40			normal 1 50	
23	9	delayed 5 30	normal 2 30	normal 2 15	normal 3 30	normal 2 15							normal 3
23	10	delayed 4 45	normal 3 35	normal 1 50	normal 3 15	normal 4 30			normal 3 35				
23	11	delayed 6 50	normal 3	normal 2	delayed 5 40	normal 2 30							
՚3	12	delayed 6 45	normal 3 25	normal 3 10	delayed 6 30								

quantitatively by the adsorption of the plasma small amounts of the factor being left sufficient to compensate the clotting defect in these samples The author tried in a few experiments to raise the amount of adsorbing barium sulphate from 20 to 50 mg without this having altered the results

In the experiments with serum it is surprising that the heated readsorbed serum was able to compensate the clotting defect in the plasma with a mild degree of Christmas factor deficiency because the Christmas factor should have been removed by the adsorption and any traces left of the factor should have been destroyed by the heating to 56° C for 30 minutes We most not forget however that serum actually is an unphysiological test substrate the composition of which differs totally from that of plasma In addition to fibrinogen prothrombin and proaccelerin it seems to lack the AHF among others The Christmas factor is apparently recovered in serum not having been consumed during the clotting We do not know for certain whether the Christmas factor in serum is identical with that in plasma That which has been demonstrated is a Christmas effect in serum

It has in fact been mentioned that serum from patients belonging to the Christmas group as well as to the group here described is able to correct the clotting defect in the patients own plasma

An intensified Christmas effect can evidently be produced during the clotting We know nothing certain of the origin of this effect It may be due to small amounts of Christmas factor or originate from the platelets Further an inhibitor may be conceived to exist which inhibits the action of the Christmas factor and which disappears during the clotting The results of the experiments with freezing may perhaps support this latter hypothesis as freezing might effect destruction or liberation of an inhibitor

On the basis of our present experience we may establish the fact that to use the thrombin generation test for classifying haemophiliacs cross tests with plasma samples lacking the AHF and the Christmas factor are required to ensure that patients classified primarily in the PTA and Hageman groups do not actually represent mild degrees of AHF and Christmas factor deficiencies

5 Deficiency of Plasma Thromboplastin Antecedent (PTA)

Rosenthal s Syndrome

In the table fig 39 are collected the patients with haemorrhagic diathesis in whose plasma the thromboplastic activity was impaired while the clotting defect was corrected whether adsorbed plasma or normal serum was added but not by adding heated readsorbed serum

This group comprised 15 patients distributed over nine families (see the table fig 39) or about 10 per cent of the whole series

As great uncertainty prevails with regard to the existence of PTA deficiency

Fig 39

Family no	Stand for pt no	Corrects the clotting defect in	
		AHF plasm	Christmas plasma
23	11	+	—
–	12	+	—
31	6	—	+
41	2	—	+
–	5	—	+
67	1	—	+
88	3	—	+
–	4	—	+
99	2	—	+
101	2	—	+
–	4	—	+
–	5	—	+
106	1	+	+
109	1	+	+
–	2	+	+
9 families	15 patients	5	13

which some writers regard as mild degrees of AHF and/or Christmas factor deficiency (*Verstraete* 1955 *Biggs & Macfarlane* 1957) there was reason to investigate whether plasma from all these patients was able to correct the clotting defect both in plasma lacking the AHF and in plasma lacking the Christmas factor this being characteristic of plasma with PTA deficiency (*Rosenthal Dreskin & Rosenthal* 1953)

The results of these tests were that plasma from ten of the 15 patients with supposed PTA deficiency was unable to correct the clotting defect in plasma lacking the AHF while plasma from two could not correct the clotting defect in Christmas plasma Plasma from three patients corrected the clotting defect in plasma lacking the AHF as well as that in plasma lacking the Christmas factor (see fig 39)

The ten plus two patients were thereafter regarded as having slight AHF and Christmas factor deficiencies respectively They have been described previously (p 81 and p 91) Two patients from the former group (family 41) were found to have a combined defect (see p 109)

Compared with the normal test substrates all the blood samples from this group had the following characteristics in common with regard to the coagulation process The recalcification time was moderately prolonged the longest being 9 min 35 sec and the shortest 4 min Five had recalcification times within the normal range

The thrombin generation likewise presented common features high thrombin concentrations having been obtained usually after a prolonged lag period

However in several instances the thrombin formation measured by the throm bin generation test must be characterised as normal (e g families 88 and 109)

Clinically the patients of this group also had certain features in common The haemophilia had in the main a fairly mild character Only two received disablement benefit seven were fully fit and two partially fit Four were chil dren They were all troubled by bleedings into the skin Twelve had haemar throses of whom five developed permanent changes Eleven had had bleedings from the gums and 7 prolonged episodes of epistaxis

The average age of this group was 25 years

True PTA Deficiency

This group comprised as stated three patients from the families 106 and 109 The patients ages ranged between 14 and 45

Symptomatology

The most important signs and symptoms are recorded in fig 40

The patients in family 109 were troubled very little by their disease They had experienced prolonged bleeding only from open injuries and following dental extractions One had had haematuria however In the third patient of this group the manifestation of the disease had been considerably more pro nounced He had had haemarthroses which had left permanent changes In this case the disease seems however to have grown considerably milder since the age of 10 The two former patients were fully fit

The Clotting Defect

The recalcification times were only slightly prolonged in plasma from the two patients with mild bleeding phenomena (5 min 33 sec and 5 min respectively) In the third the time was definitely prolonged 9 min 35 sec (See the table fig 41)

In agreement with this the thrombin generation test revealed a practically normal thrombin generation in the two patients from family 109 The lag period was just over 4 minutes thus being only slightly prolonged The maximal thrombin concentration was very high and the inactivation of the thrombin formed occurred at a normal rate In the third patient (family 106) the thrombin generation proceeded at a far slower rate The lag period was about 6–7 min utes

A normal thrombin generation was in all three cases obtained after addi tion of adsorbed bovine plasma or serum but not after addition of heated readsorbed serum In two of the samples the effect of adding readsorbed serum was investigated The thrombin generation became normal

Fig
PTA

Family	Patient no	Age	First manifest (year)	Mild r with ncr years	Haemarthroses	Permanent joint deformation	Bleeding in skin	Bleeding in muscles	Gastrointestinal haemorrhages	Urinary tract haemorrhages
106	1	14	0	10	+	+	+	—	—	—
109	1	45	2	?	—	—	+	—	—	—
—	2	28	?	?	—	—	+	—	—	+
	3 patients			1	1+	1+	3+	0	0	1+

in both After the plasma had been stored with its normal content of platelets at $-20°$ C the thrombin generation became normal in all three cases In one case the thrombin generation in platelet poor plasma stored at $-20°$ C was also investigated (family 109 no 2) The platelet content was 3000 per cubic millimetre of plasma The thrombin generation became normal In one sample the effect of the patient's own serum on the thrombin generation was investigated (family 106) The thrombin generation became perfectly normal recalcification time 4 min 10 sec

In the cross tests the plasma samples from these three patients normalised the thrombin generation in plasma lacking the AHF as well as in plasma lacking the Christmas factor

There were no signs of a haemorrhagic diathesis among the female members of these two families In one the mother of the patient in family 106 the clotting time had been investigated previously and found to be normal (see chapter XIII)

Fig 41

Thrombin generation test and recalcification times in plasma with PTA deficiency

Family	Patient no	Added: Physiological saline 0 2 ml	Adsorbed bo plasma 0 2 ml	Serum 0 2 ml	Heated adsorbed serum 0 2 ml	Readsorbed serum 0 2 ml	Thrombin generation from plasma	Added with platelets	Thrombin generation from platelet poor plasma
106	1	delayed 9 35	normal 3	normal 3 ?0	delayed 6 50	normal 4 18	normal 3 45		
109	1	delayed 5 33	normal 3 50	normal 2 38	delayed 5 25	normal 3 3	normal 4 25		
—	2	delayed 5	normal 3 15	normal 2	delayed 4 45		normal 2 10	normal ?	normal 3 25

40

deficiency

G g l h mo rrh ges	Bleed g al ervou y t m	Ep t u	Lo r resp t	Oth r	F t t wo k	Occ pat n	Rec l ficat on-time
+	–	+	–	–	partially	schoolboy	9 35
+	–	–	–	–	fully	small holder	5 33
+	–	–	–	–	fully	farmer	5
3+	0	1+	0	0	2		

Discussion

Rosenthal Dreskin & Rosenthal (1953) diagnosed their first case of PTA deficiency by determining the serum prothrombin time and the recalcification time in plasma and blood They found that plasma lacking the plasma thromboplastin antecedent could normalise the clotting defect in plasma and blood lacking the AHF and the Christmas factor The clotting defect in PTA-deficient blood could be corrected by adsorbed plasma as well as by normal serum Adsorbed serum also contains PTA (*Rosenthal* 1955) The results of the present investigation are in fair agreement with these observations

Ramot Angelopoulus & Singer (1955 a) found three patients with PTA deficiency two women and one man The clotting time for blood from these likewise became normal after addition of either adsorbed plasma or serum Similar results were achieved after addition of adsorbed plasma from a patient with AHF deficiency which contains neither AHF nor Christmas factor and of serum from a patient with Christmas disease which likewise is supposed to lack the AHF as well as the Christmas factor However we cannot exclude the possibility that adsorbed serum contains a factor possessing Christmas activity Conversely adsorbed plasma and serum from a PTA patient were found by these authors to eliminate the clotting defect and normalise the serum prothrombin time in plasma from patients with AHF deficiency and Christmas factor deficiency respectively

One of the Danish patients had a rather pronounced clotting defect and had previously displayed rather severe bleeding phenomena Such are unusual in PTA patients though they have been described by *Oehme & Hagette* (1955)

Ramot Angelopoulus & Singer (1955 a) point out as characteristic of the PTA patients that the clotting times vary and that the disease has different clinical manifestations within the same family

Fig

Hageman

Family	Patient no	Age	First manifest. (year)	Mild with incr years	Haemarthroses	Permanent joint deformation	Bleeding in skin	Bleeding in muscles	Gastrointestinal haemorrhages	Urinary tract haemorrhages
8	3	52	7	30	–	–	+	–	–	+
36	4	28	1/2	18	+	–	+	–	–	–
51	4	24	2	17	+	–	+	–	–	–
–	5	26	26		–	–	–	–	–	–
56	3	27	7	14	–	–	+	–	–	–
63	2	64	10	30	–	–	+	–	+	–
80	2	1	1/2		+	–	+	–	–	–
88	5	16	2		+	–	+	–	–	–
90	1	14	1	12	+	–	+	–	–	–
112	1	47	10		–	–	+	–	–	–
–	2	15	1		+	–	+	–	–	–
9 families	11 patients				6+	0	10+	0	1+	1+

6 Deficiency of the Hageman Factor

Blood samples from haemophilic patients with an impaired thromboplastic activity were at first classified in the group of the Hageman trait if the thromboplastic activity became normal after addition of adsorbed bovine plasma or after *addition of heated readsorbed* serum

These requirements were fulfilled by 18 samples from patients belonging to the families 8 18 23 36 51 56 63 80 88 90 92 and 112 This was a surprisingly large number Cross tests with plasma samples lacking the AHF and the Christmas factor showed however that four of these patients had a slight Christmas factor deficiency (families 8 and 23) and three a slight AHF *deficiency (families 18 88 and 92) Eleven patients were left from* nine families who were classified in the group of Hageman defects as blood samples from these in addition to fulfilling the above requirements had a normal thromboplastic activity in mixtures with plasma samples lacking the AHF and samples lacking the Christmas factor Looking at the group as a whole (all 18) the different forms are indistinguishable clinically These were mild cases of haemophilia All the adults were fully fit the recalcification times were only moderately prolonged and thrombin formation took place to varying extents but was delayed

Symptomatology of the True Hageman Trait

*The most im*portant clinical data are shown in the table fig 42 The patients ages ranged from 1 to 64 years averaging 29 None of the patients had had

42

trait

G g l h mo- rrh ges	Bleeding in ce r l nervo syst m	Ep ta	Lowe p t a t	Oth	F t t wo k	Occ p t o	Recal ficat n t m
+	–	+	–	–	*fully*	*farm owner*	*4 10*
+	–	–	–	–	fully	telegraph operator	5 35
+	–	+	–	–	fully	clerk	5 50
+	–	–	–	–	fully	student of engineering	4 25
+	–	+	–	–	fully	pianist	6 20
+	–	+	–	–	fully	debt collector	6 45
+	–	–	–	–			5 53
+	–	+	–	–	fully	printer s apprentice	3 50
+	–	–	–	–			8
+	–	–	–	–	*fully*	*farm owner*	*5 30*
+	–	–	–	–	fully	schoolboy	5 30
11+	0	5+	0	0			

abnormal bleeding phenomena at birth The first manifestation of the disease occurred between the ages of 6 months and 26 years Five were over 7 years old at the first sign of a haemorrhagic diathesis

All the patients had had bleedings from the gums, either in association with dentition or following dental extraction Ten had had bleedings into the skin and six haemarthroses none of which had resulted in permanent changes Five had had prolonged episodes of epistaxis and one had had melaena

All the adults were fully fit The children were not particularly troubled by their disease They could even attend the gymnastic lessons at school

The Clotting Defect

The clotting times in dilute plasma ranged from 3 min 50 sec to 8 min

A characteristic feature of the thrombin generation in the plasma of these patients was that a lag period of 5–6 minutes was usually followed by a pronounced rise of the thrombin concentration which often reached high val ues

The table fig 43 gives a survey of the most important experiments made with plasma from these patients The figures indicate the recalcification times

Various interesting observations are recorded in the table 1) in five cases platelet suspensions were prepared from the patients own plasma The platelets were washed and then added again to the plasma A normal thrombin genera tion was obtained throughout In one instance (family 63 no 2) the influence of the platelet wash water on the thrombin generation in normal plasma was investigated The wash water was seen to have no influence 2) In eight plasma

Fig 43

Hageman trait

I c bat on m ture						F oz plat l t ch pla ma	Added froz n pl t let t pen on	Added fr h platel t s spe s on	F oz n pl t let poor pla ma
ml of patient plasma		1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0
ml of adsorbed bovine plasma			0 2							
ml of serum					0 2					
ml of readsorbed serum										
ml of heated readsorbed serum				0 2						
ml of normal plasma										
ml of 0 85 saline		1 0	0 6	0 8	0 8		1 0	1 0	1 0	1 0
ml of $CaCl_2$		1 0	1 2	1 0	1 0	1 0	1 0	1 0	1 0	1 0
ml of own washed platelets						1 0				
Family	*Patient no*									
8	3	delayed 4 10	normal 3 21	normal 2	normal 3		normal 3 50	normal 4 10	normal 2	normal 2 5 (9000)
36	4	slow 5 35	normal 3 40	normal 4	normal 1 40	normal 4 15	normal 4	normal 3	poor 5	normal 3 (9000)
51	4	delayed 5 50	normal 2 50	normal 2 45		normal 2 30				normal 2 45
51	5	delayed 4 25	normal 3 35	normal 3 76	normal 1 19		normal 3 40	normal 3 36		
56	3	delayed 6 _0		normal 4				normal 4 15		
63	2	delayed 6 45	normal 3 30	normal 3 25	normal 2 30	normal 2 45	normal 1 40			normal 2 45 (8000)
90	2	delayed 5 53	normal 4	normal 2 50	normal 1 33		normal 2 30	poor 5	poor 6 17	poor 4 30 (6000)
88	5	delayed 5 45	normal 3	normal 3 45	normal 1 55		normal 2 30		normal 2 30	normal 2 50
90	1	poor 8	normal 2 45	normal 2 40	normal 1 30		normal 2 25			normal 3 50 (25000)
112	1	delayed 5 30	normal 3 15	normal 3		normal 2 30	normal 1 42			normal 2 20 (3000)
112	2	poor 5 30	normal 1 45	normal 1 40		normal 1 10	normal _30			normal 5 (_000)

samples from these patients the thrombin generation and the recalcification time were investigated after the plasma samples had been stored with their normal content of platelets at −20° C The thrombin generation became normal in all eight cases 3) In eight cases the influence of freezing on platelet poor plasma was studied In these experiments the numbers of platelets ranged from 5000 to 25 000 The thrombin generation became normal in seven samples In the eighth (family 80 no 2) the thrombin generation was poor but a fall had occurred in the recalcification time from 5 min 53 sec to 4 min 30 sec The influence of freezing on the coagulability of plasma lacking the Hageman factor thus seems to be fairly independent of the number of platelets

The influence of freezing on platelet *free* Hageman plasma has not been investigated *A priori* we should not expect the thrombin generation to become normal in this plasma as it would not become so in platelet free normal plasma after freezing the platelets being necessary for the normal coagulation 4) In three cases small amounts of fresh normal platelet suspension were added In one the thrombin generation became normal 5) Frozen platelet suspension was added to four samples Three of these became normal with regard to both thrombin generation and recalcification

All these plasma samples normalised the thrombin generation in plasma with AHF deficiency and Christmas factor deficiency

The patients own serum corrected the clotting defect in their own plasma (see family 8 no 3)

Discussion

It has been shown above that the clotting defect of plasma samples lacking the Hageman factor can be corrected in the following ways

1) by adding adsorbed normal plasma
2) by adding heated readsorbed serum (and untreated serum as well as own and normal serum)
3) by freezing the plasma with its normal content of platelets
4) by freezing vigorously centrifuged i e platelet poor plasma (seven out of eight cases)
5) by adding the patients own washed platelets to their substrate plasma
6) by adding a normal frozen platelet suspension (three out of four cases)

The facts stated in items 1) and 2) are in agreement with previous information on the coagulability of Hageman plasma (*Ratnoff & Colopy* 1955 *Frick & Hagen* 1956)

The results of investigating the influence of freezing on the thrombin generation in plasma with this defect have been mentioned previously (*Sjølin* 1956 b 1957 b d) These are not specific for Hageman plasma a similar influence having been observed though less frequently in plasma lacking the AHF the Christmas factor and the PTA (see pp 80 89 98) As normalisation of the

thrombin generation may be seen in plasma with a normal platelet content as well as in platelet poor plasma the possibility might be conceived that the freezing does not act *via* the platelets, unless the thromboplastin formation can be normalised by liberation of only very little platelet factor

Results like those of experiments with washed platelets where the patient's own platelets suspended in saline normalised the thrombin generation have been achieved and described previously for both horse and man (Sjølin 1957 a d) The same has been observed in plasma with slight Christmas factor deficiency (see p 93) It is hardly due to removal of an inhibitor because the platelet wash water does not inhibit the thrombin formation in normal plasma Neither can it be a question of transfer of Hageman factor, which is adsorbed on the surface of the platelets as the platelets originate from the patient plasma

If we suppose that the object of the Hageman factor is to render the platelets labile and consequently to accelerate the liberation of the elements of the platelets contributing towards the plasma thromboplastin, this might perhaps, explain the effects of freezing and of washing the platelets Both processes presumably have such a traumatising effect on the platelets that their content of thromboplastic factor is likely to be liberated These processes might be conceived to act as substitutes for the Hageman factor which in the remaining experiments was added with adsorbed plasma or heated readsorbed serum This may also explain the courses of the thrombin generation curves High though delayed thrombin concentrations usually occur spontaneously These are so high that the amounts of thrombin formed are normal

By promoting the disintegration of the platelets in one of the above ways the curve is shifted to the left The thrombin generation will now set in at a normal point of time the clotting defect has been compensated

Fig

Antihaemophilic factor plus

(Combined

Fam ly	P tient no	Age	F rst ma fest (yea)	Mild r w th er years	Haemar throses	Pe m t) m deforma t	Bleed g sk n	Bleeding muscles	G st intest l haemo-rrh ges	U ary t ct haemo-rrhages
1	4	46	1	–	+	+	+	+	+	+
–	9	6	0	–	–	–	+	–	–	–
40	11	29	3	–	+	+	+	+	–	+
42	4	29	6/12	–	+	+	+	–	–	+
58	2	21	1	–	+	+	+	+	+	+
95	1	43	0	–	+	+	+	+	+	–
5 families	6 patients			0	5+	5+	6+	4+	3+	4+

Plasma from patients with the stated Hageman defects was as stated able to normalise the thrombin generation in plasma lacking the AHF and in plasma lacking the Christmas factor The influence of the plasma on PTA deficient plasma was investigated in one case (family 109, no 2) Here too the thrombin generation and the recalcification time became normal *This is* not quite in agreement with *Frick & Hagen* (1956) who mention that Hageman plasma is unable to normalise the poor prothrombin consumption in plasma lacking the AHF and PTC, and particularly in plasma lacking the PTA On the other hand to single out a new coagulation factor one must *require that plasma lacking this new factor is able to correct the clotting defect* in plasma samples with other defects unless the presence of multiple defects can be evidenced

The author could detect no clotting defect in the mothers of two of the the Hageman patients investigated

An asymptomatic Hageman trait has been described previously in this country (*Sjølin* 1957 b) but is not included here at it is not to be regarded as a form of haemophilia

7 Combined Clotting Defects

Two groups have been referred to the combined clotting defects 1) Deficiency of the antihaemophilic factor plus Christmas factor (combined haemophilia) and 2) *a not previously described group characterised by the somewhat lengthy* name of deficiency of the antihaemophilic factor plus freezing/serum factor The latter designation probably covers a slight Christmas factor deficiency

44

Christmas factor deficiency
haemophilia)

G g l haemo-rrh ges	Bleed g in ce t l ne y t m	Ep taxi	Low esp t t	Oth	F t t w k	Occ p t	Recal fica n-t m
+	–	+	–	–	–	pensioner	16 30
–	–	–	–	+	–		15
+	–	–	–	–	(+) (D B)	motor-car dealer	10 30
+	–	+	–		– (D B)		15
+	–	+	–	–	– (D B)		15
–	–	+	–	–	– (D B)		12 30
4+	0	4+	0	1+			

Combined Haemophilia AHF plus Christmas Factor Deficiency

A diagnosis of combined haemophilia (AHF plus Christmas factor deficiency) was made in the cases where the clotting defect of plasma from haemophiliacs could only be corrected by simultaneous addition of adsorbed bovine plasma and normal human serum Simultaneous addition of adsorbed bovine plasma and heated, *readsorbed serum did not correct this clotting defect*

The present investigation revealed combined haemophilia in six patients belonging to the families 1 40 42 58, and 95 The most important clinical data are recorded in the table fig 44 The patients ages ranged from 6 to 46 years averaging 29

Symptomatology

In this group the first sign of a haemorrhagic diathesis occurred very early, on an average at the age of 11 months Two presented abnormal bleeding phenomena at birth

All the patients had had bleedings into the skin Second in frequency were haemarthroses which had occurred in five adults and had left permanent changes in all five Four had had intramuscular haemorrhages haematuria gingival haemorrhages and epistaxis Three had had gastro intestinal haemorrhages There had been no bleedings in the central nervous system or the respiratory tract

The bleeding tendency had decreased in none of the patients of this group Four received disablement benefit and a fifth (family 1 no 4) had been granted a pension This patient who in the author s opinion was the most severely affected of the whole Danish series was also disabled

This patient was the only one of the group who had previously been able to support himself and his family The patients with combined haemophilia are thus exceedingly troubled by their disease

The Clotting Defect

The recalcification times in dilute plasma from these patients was greatly prolonged ranging between 10 min 30 sec *and 16 min 30 sec (See the* table fig 45)

The thrombin generation was very poor in all cases practically no thrombin having been formed within the period of testing

In no case did the thrombin generation become normal till after simultaneous addition of adsorbed bovine plasma and normal serum Addition of adsorbed bovine plasma alone effected in all cases normalisation of the recalcification time but only little improvement of the thrombin generation In five cases addition of serum alone normalised the recalcification time but in no case the thrombin *generation*

Fig 45

Antihaemophilic factor plus Christmas factor deficiency

(Combined haemophilia)

I b t m tur								F o pl t let-rich plasma
ml of patient plasma	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0
ml of adsorbed bovine plasma		0 2		0 2	0 2		0 2	0 2
ml of serum			0 2	0 2				
ml of readsorbed serum					0 2			
ml of heated readsorbed serum							0 2	
ml of normal plasma						0 2		
ml of 0 85 / saline	1 0	0 6	0 8	0 4	0 4	0 6	0 4	0 6
ml of CaCl	1 0	1 2	1 0	1 ?	1 2	1 2	1 2	1 2

Family	*Patient no*								
1	4	poor 16 30	some improvement 3 25	some improvement 4 30	normal . 0	some improvement 3 36	normal 3 30		
1	9	poor 15	some improvement 4 45	some improvement 4 45	normal 3 35	some improvement 3 0			
40	11	poor 10 30	some improvement 4 15	slight improvement 3 45	normal 3		normal 2 45	some improvement	some improvement 4 30
42	4	poor 15	some improvement 3 15	slight improvement 5	normal 2 33	some improvement 5 23			some improvement 5 40
58	2	poor 15	some improvement 4 45	slight improvement 6 30	normal 3				some improvement 5
95	1	poor 12 30	some improvement 3 15	slihgt improvemnet 2 45	normal 2 25		normal 2 30		some improvement 4

Discussion

Only six cases of combined haemophilia have been described so far (*Soulier & Larrieu* 1953 *Verstraete & Vandenbroucke* 1955 *Hill & Speer* 1955 *Sjølin 1957* c) Two of the six patients described above have been reported previously (*Sjølin* 1957 c)

It is remarkable that about 4 per cent of the total series under review had combined haemophilia which according to *Verstraete & Vandenbroucke 1955) is an extremely rare phenomenon*

Biggs & Macfarlane (1957) found no instances among their 164 haemophilic patients

Three of the patients described here belonged to families in which no other cases of haemophilia were known (*families 42 58 and 95) Two were* found in a family (family 1) in which a third living haemophiliac with a fairly mild bleeding tendency had AHF deficiency alone and in which haemophilia had occurred previously One belonged to a family (family 40) in which there had previously been many haemophiliacs

All the patients described here were greatly troubled by their disease which seems to have persisted unchanged In these cases there seems to be agreement between the clotting defect and the symptomatology

The diagnosis on the basis of the thrombin generation test using normal test substrates caused no difficulty in these cases In one case other haemophilic plasma samples with known defects were investigated in the presence of fresh plasma from a patient of the group under review (see however also patient no 11 family 2) In this single case (family 42) neither plasma lacking the antihaemophilic factor nor plasma lacking the Christmas factor could render the thrombin generation normal If the plasma sample from the patient in family 42 had been frozen down it could have normalised the thrombin generation in plasma with known Christmas factor deficiency Evidently as also mentioned in the section on Christmas disease freezing causes an *activation* of the Christmas factor so that the double defect is masked

Frozen plasma samples from the other five patients of this group likewise normalised the thrombin generation in plasma from patients suffering from *Christmas* disease (families 4 14 and 85) The Christmas defect in these patients with double defect may not be complete

These patients were classified in a separate group mainly because of the characteristic reaction of the plasma samples to the test substrates

One patient (family 42) was investigated twice at an interval of 2 years On the second testing the clotting defect seemed to be less pronounced and able to be corrected by adsorbed bovine plasma alone Added serum also accelerated the thrombin generation however though far less so

The favourable response to bovine plasma alone may be due to presence of a small but sufficient amount of Christmas *factor to compensate this defect*

The results of the recalcification time determinations in this patient suggest a spontaneous variation of the clotting defect

Soulier & Larrieu (1953) diagnosed their cases by clotting time determination *Verstraete & Vandenbroucke* (1955) by thromboplastin generation test and *Hill & Speer* (1955) by prothrombin consumption test The latter writers had previously classified their two patients in the group of AHF deficiency

It is surprising that the average age for this greatly affected group of patients was as high as 29 This is presumably due to the modern improved transfusion service as well as to the fact that such patients are confined to bed for long periods thus being exposed to fewer injuries than the others

8 Deficiency of the Antihaemophilic Factor plus Freezing/Serum Factor (Christmas Factor)

In the section on classical haemophilia (deficiency of the antihaemophilic factor) a group was mentioned in which adsorbed bovine plasma alone normalised the thrombin generation only when the patient s plasma had been frozen (p 75) The clotting defect of fresh patient plasma could also be corrected by adding heated readsorbed serum simultaneously with the bovine plasma

This group has been given the neutral name of deficiency of the antihaemophilic factor plus freezing/serum factor to avoid introduction of new syndromes (see p 111)

The group comprised 18 patients belonging to the families 21 25 35 39 41 52 59 74 75 96 101 and 108 The ages ranged from 7 to 64 years averaging 28

Symptomatology

In 12 patients the first sign of a haemorrhagic diathesis occurred within the first year of life in two within the second in three at the age of 3 4 and 7 respectively and in one allegedly not till the age of 10 (see the table fig 46)

All the patients of this group had had bleedings into the skin and haemarthroses which latter had left permanent joint deformities in 15 cases The three without permanent changes were 15 years of age or younger Then followed in decreasing order of frequency bleeding from the gums (14) haematuria (11) epistaxis (10) intramuscular bleedings (6) and gastro intestinal bleedings (4)

A single patient had had haemopericardium apparently spontaneous and another prolonged haemorrhage after paracentesis None had experienced bleedings in the central nervous system nor in the lower respiratory tract Five declared that the disease had grown milder with increasing years of whom three fixed the time for this at between 15 and 21 years of age

Three patients of this group received disablement benefit Four were unfit for work Four others were rather crippled of whom two were nevertheless

Discussion

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It is remarkable that about 4 per cent of the total series under review had combined haemophilia which according to *Verstraete & Vandenbroucke* 1955) is an extremely rare phenomenon

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All the patients described here were greatly troubled by their disease which seems to have persisted unchanged In these cases there seems to be agreement between the clotting defect and the symptomatology

The diagnosis on the basis of the thrombin generation test using normal test substrates caused no difficulty in these cases In one case other haemophilic plasma samples with known defects were investigated in the presence of fresh plasma from a patient of the group under review (see however also patient no 11 family 2) In this single case (family 42) neither plasma lacking the antihaemophilic factor nor plasma lacking the Christmas factor could render the thrombin generation normal If the plasma sample from the patient in family 42 had been frozen down it could have normalised the thrombin genera tion in plasma with known Christmas factor deficiency Evidently as also mentioned in the section on Christmas disease freezing causes an activation of the Christmas factor so that the double defect is masked

Frozen plasma samples from the other five patients of this group likewise normalised the thrombin generation in plasma from patients suffering from Christmas disease (families 4 14 and 85) The Christmas defect in these patients with double defect may not be complete

These patients were classified in a separate group mainly because of the characteristic reaction of the plasma samples to the test substrates

One patient (family 42) was investigated twice at an interval of 2 years On the second testing the clotting defect seemed to be less pronounced and able to be corrected by adsorbed bovine plasma alone Added serum also accelerated the thrombin generation however though far less so

The favourable response to bovine plasma alone may be due to presence of a small but sufficient amount of Christmas factor to compensate this defect

46

free.ing/serum factor

Gingival haemo-rrhages	Bleed g ce t al rvo yst m	Epist	Lower esp t act	Oth	F t to w k	Occup t o	Rec lc fic tio t m
+	−	+	−	+			13 10′
+	−	+	−	−	-	D B	16
+	−	+	−	−	partially	herdsman	15
+	−	+	−	−	partially	assistant	8 45
+	−	+	−	+	fully	woodcutting machinist	11 45
+	−	+	−	−	partially	music teacher	12 40
+	−	−	−	+	-		20
−	−	+	−	−			8 30
+	−	+	−	−	-	D B (shoemaker)	8 25
+	−	+	−	−	-	D B	8 40
+	−	−	−	−	-		10 30
+	−	+	−	+	partially	business man	16 50
−	−	−	−	−	partially	woodcutting machinist	12 45
+	−	−	−	−			11 20
−	−	−	−	−			17
+	−	−	−	−			9 50′
+	−	−	−	−	fully	farm owner (soldier)	6 45
−	−	−	−	+	partially	bookbinder	6
14+	0	10+	0	5+			

with its normal platelet content at −20° C resulted in normal thrombin genera tion and recalcification time In the 13th case it effected rapid formation and inactivation of thrombin but the maximum thrombin concentration was low In 12 cases the same test was made with vigorously centrifuged plasma In ten of these the thrombin generation became normal Among these was the patient whose platelet-containing plasma had not been investigated after freez ing (family 39 no 23)

In ten cases adsorbed bovine plasma and heated readsorbed serum were added simultaneously The thrombin generation and the recalcification time became normal in all ten cases

Discussion

The first question suggesting itself is whether one may be justified in differen tiating this group from that of pure AHF deficiency The possibility might be conceived that only the severest degrees of AHF deficiency were collected in this group This is not so however Two of the patients were troubled but little by their disease one of whom had a fairly good though delayed thrombo-plastic activity in the plasma In a few instances (e g family 59 no 2 and

Fig

Deficiency of AHF +

Family	Patient no	Age	First manif (years)	Mdd r w th i y a s	Haemarthroses	Permanent joint deformations	Bleeding in skin	Bleeding in muscles	Gastro intestinal haemorrhages	Urinary tract haemorrhages
21	4	15	5/12	—	+	+	+	—	+	+
25	3	23	3	—	+	+	+	—	—	+
–	4	17	0	—	+	+	+	—	—	+
–	5	15	7	—	+	—	+	—	—	—
35	6	50	2	+	+	+	+	+	—	+
39	21	34	1	20	+	+	+	+	+	+
–	*23*	*15*	*10/12*	—	+	+	+	+	—	—
–	26	7	2/12	—	+	—	+	—	—	—
41	2	35	1	—	+	+	+	+	+	+
–	5	19	0	—	+	+	+	—	—	—
52	3	35	4	—	+	+	+	+	—	+
59	1	64	1	—	+	+	+	+	—	+
–	2	50	1	+	+	+	+	—	—	+
74	2	12	3/12	—	+	+	+	—	—	—
75	1	10	5/12	—	+	—	+	—	—	—
96	1	12	0	—	+	+	+	—	+	—
101	3	64	10	+	+	+	+	—	—	+
108	1	21	18/12	15	+	+	+	—	—	+
12 families	18 patients			5+	18+	15+	18+	6+	4+	11+

able to manage as business men while one earned a living as a music teacher and one as a bookbinder Two were fully fit hardly feeling troubled by their disease One of these had done his military service The remainder were children of whom one was already greatly crippled

The Clotting Defect

The recalcification times in dilute plasma from these patients were fairly long ranging between 4 min 30 sec (family 74) and 20 min 11 were over 10 min

The thromboplastic activity was very poor in 17 out of the 18 plasma samples In one (family 101 no 3) a very high thrombin concentration was obtained but only after a greatly prolonged lag period

In the substitution experiments it was characteristic that addition of adsorbed bovine plasma alone produced a fall in the recalcification time but only slight improvement of the thrombin generation Similar reactions were found to serum added alone

In five cases normal fresh plasma was added This gave in all five samples a perfectly normal recalcification time and thrombin generation

In 12 out of 13 cases addition of adsorbed bovine plasma to plasma stored

9 Miscellaneous

This group comprises patients with clotting defects which are classifiable in none of the above mentioned groups as well as patients previously diagnosed as haemophiliacs but in whom the present investigation revealed no clotting defect

Slight Deficiency of the Antihaemophilic Factor plus Christmas Factor

Patient no 1 in family 77 has a combined defect presumably a slight AHF plus Christmas factor deficiency He might perhaps rightly have been clas sified in that group As however the symptomatology and the results of the haematological investigations differed from those for the six patients described under that heading it was preferred to mention this patient separately (see the tables figs 47 and 48)

Symptomatology

The patient was previously troubled by haemarthroses which had left perma nent changes In addition he had been liable to epistaxis subcutaneous haema tomas bleeding from minor lesions and from the oral mucosa The disease has grown milder the last few years He is now being trained as a telegraph operator

The Clotting Defect

The recalcification time was only moderately prolonged (see the table fig 48) 5 min 40 sec The thrombin generation test showed considerable though delayed thrombin generation The clotting defect was corrected by adsorbed bovine plasma serum heated readsorbed serum and own washed platelets Freezing caused acceleration of the thrombin generation but the concentra tions obtained were fairly low

47

laneous

G g l h mo- h ges	Bleeding ce l rv y t m	Ep t is	Lo er p tr t	O h	F t t w k	Occ p t	Rec l fi t t m
+	−	+	−	−	−		20
−	−	+	−	+	partially	Telegraph operator	5 40

family 25 no 3) the author added adsorbed bovine plasma in double the usual amount to patient plasma without the thrombin generation becoming normal This militates against the hypothesis of an isolated severe AHF defect

The fact that addition of adsorbed bovine plasma as well as heated readsorbed serum normalised the thromboplastic activity might suggest a double defect with deficiency of both the AHF and the Hageman factor This is also unlikely because the Hageman factor is present in adsorbed bovine plasma

The factor which the plasma of these patients lacks besides the AHF and which has been termed the freezing/serum factor is present in normal plasma and normal serum It is apparently not adsorbed from serum on barium sulphate and it stands heating to 56° C for half an hour On the other hand it is removed from bovine plasma by adsorption with barium sulphate so accordingly it cannot be identical with the Hageman factor

The freezing/serum factor can apparently be substituted by freezing and *subsequent thawing of the plasma What happens by this process is unknown,* except that the platelets are damaged However the activation" of this factor seems to be largely independent of the number of platelets though none of the investigated plasma samples were platelet free

It is possible that serum and the platelets contain factors capable of neutralising naturally occurring inhibitors (*Nilsson & Wenckert*)

Another possibility is a combination of AHF deficiency and a very slight Christmas factor deficiency This hypothesis is supported by the fact that the thrombin generation did not become normal in the cases where cross tests were made between fresh plasma from these patients and other plasma samples lacking the Christmas factor (e g family 52 no 3 and family 74 no 1) It has been shown previously that the Christmas defect can in many cases be compensated by freezing the plasma We also experienced that in some cases within the group of slight Christmas factor deficiency the clotting defect could be compensated by adding heated readsorbed serum

Clinically the majority of such patients are probably indistinguishable from patients lacking the AHF

Fig.

Miscel

Family	Patient no	Age	First manifest (years)	Milder with ... years	Haemarthroses	Persistent joint deformation	Bleeding in skin	Bleeding in muscles	Gastrointestinal haemorrhag	Urinary tract haemorrhages
70	1	16	3/12	–	+	+	+	+	–	–
77	1	21	1	20	+	+	+	–	–	–

Neither plasma from a patient with Christmas factor deficiency nor plasma lacking the antihaemophilic factor normalised the thrombin generation or the recalcification time when added separately whereas when added simultaneously the thrombin generation became normal

The experiments with normal substrates suggested deficiency of the Hageman factor The reaction to the freezing test was not quite characteristic of the Hageman defect The results of cross tests with plasma samples having known defects argued in favour of AHF plus Christmas factor deficiency though probably of a slight degree

Thromboplastin Inhibitor

One patient within the total Danish series of haemophiliacs was found to have an inhibitor in the blood (family 70 no 1)*) This patient had very pronounced manifestations of the disease including large haemarthroses and intramuscular haemorrhages He claimed to have noticed that the bleeding tendency increased after transfusions (see fig 47)

The clotting defect was very severe with a greatly prolonged recalcification time and totally absent thrombin generation which could be normalised neither by adsorbed bovine plasma serum or plasma nor by plasma from patients with AHF deficiency or Christmas factor deficiency (see fig 48)

The thrombin fibrinogen reaction was normal Thus there was no qualitative fibrinogen defect nor any sign of a thrombin inhibitor (see p 279)

The patient s plasma prolonged the clotting time in normal plasma (see p 279)

This argues in favour of the presence of a plasma thromboplastin inhibitor

By adding decreasing amounts of tissue thromboplastin to the patient s plasma low thromboplastin concentrations were found to cau e a sudden prolongation of the clotting time whereas the clotting time of the control plasma was fairly independent of the thromboplastin concentration No further identification of the demonstrated thromboplastin inhibitor had been undertaken at the time when this was written

Haemophilic Patients With No Demonstrable Thromboplastic Defect and With Prolonged Freedom from Abnormal Bleeding Phenomena

This group comprised three patients who in addition to having a normal coagulation system had been completely free from any abnormal bleeding phenomena through several years (see the tables Figs 49 and 50) These patients were all included in *Andreassen* s series having previously had signs of haemophilia and a prolonged clotting time

*) After the conclusion of this work an inhibitor was detected in a boy who had previously been found to lack the Christmas factor

Fig 48

Miscellaneous

Fam ly	pat t n	Th mb g at o	b + ad plasma	+ rum	+ ead se um	+ h ated eads serum	+ f o e plat l t h pla ma	+ Ch stma pl ma	+ AHF pl ma	+ own washed plat l ts	+ no mal plasma	+ pl t l t susp ns on
70	1	poor 20	poor 5	poor 4				poor 5	poor 7		poor 7	poor 15
77	1	delayed 5 40	normal 3 45	normal 3		normal 5	normal 2	delayed 5 15	delayed 6	normal 4 38		

Fig 49

Thrombin generation in haemophilic patients with no demonstrable clotting defect

		Added to AHF pla a	Add d to Ch lmas pl ma	Proth mb n t me	Pl lel ts per mm of pla m
Family 24 no 4	normal 3 5	normal 4	normal 4 10	normal	272000
Fimily 29 no 2	normal 2 45	normal 3 30	normal 2 30	normal	3? 000
Family 56 no 4	normal 4	normal 4		normal	235000

developed signs of haemophilia with epistaxis ecchymoses bleedings from the gums and a single haemarthrosis *Andreassen* as well as the author previously found a slightly prolonged clotting time for the patient s blood using Burker s method 12–15 min and 11–12½ min respectively

Repeated subsequent investigations revealed no clotting defect The patient has presented no signs of a haemorrhagic diathesis for 6 years Only by the time this work was concluded Hageman factor deficiency was finally demon strated That is the reason why he has been investigated in connection with the above group

Yet another case (family 51 no 5) will be reported in relation to this group The patient had had no haemophilic signs until at the age of 26 a dental extraction was followed by secondary haemorrhage The thrombin gen eration test revealed a somewhat prolonged lag period which was shortened by adding heated readsorbed serum to the plasma This mild doubtful clotting defect was not found again on subsequent testing and the patient has had no signs of haemophilia since

It is a well known fact *(Andreassen)* and has been confirmed previously in the present work that the bleeding tendency may abate in the course of years as a rule between the ages of 20 and 35 Haemophiliacs may however not only become free from signs and symptoms but also obtain a normal

50

demonstrable clotting defect

G g al h m h ges	Bleed g t l ry y t m	Ep t	Low p t act	O h	F t t w k	Occ p t on	Rec l fi at t m
–	–	+	–	–	fully	shop assistant	3 5
+	–	–	–	–	fully	messenger (+ soldier)	2 45
–	–	+	–	+	fully	undergraduate	4

The first patient of this group (family 24 no 4) previously had bleedings in the skin and episodes of epistaxis His maternal grandfather who unfor tunately lives abroad suffers from a mild degree of haemophilia on which account he was exempted from military service A brother of the latter is a haemophiliac and has become disabled owing to haemarthroses *Andreassen found a clotting time of 17 minutes in the patient as well as in his mother*

Thus the past history as well as the inheritance and the results of previous haematological investigations go to show that the *patient is a haemophiliac*

The patient is now free from any haemophilic manifestations is fully fit and has no demonstrable clotting defect

The second patient of this group (family 29 no 2) first manifested a bleeding tendency at the age of 10 years He had had gingival and intra muscular haemorrhages haematuria and haemarthroses *Andreassen* found pro longed clotting times in the patient s blood (25 min) and in that of his mother *(14½ min) There were no other cases of haemophilia in the family* The patient has displayed no signs of the disease for the past 20 years He has done his military service and he now has a job as a messenger

The present investigation revealed no clotting defect

The third patient belongs to family 56 (no 4) of whose members a brother and the maternal grandfather are said to have had a mild degree of haemophilia The patient himself had previously had prolonged bleedings from the lip oral mucosa and nose *Blood from this patient* too as well as from his mother had previously shown a prolonged clotting time 16 and 12¹/ min respectively *(Andreassen)*

The patient has displayed no abnormal bleeding phenomena since the age of 12–14 and the present investigation revealed no clotting defect

One of the patient s brothers who likewise is a haemophiliac has had no signs of the disease since the age of 15 The author demonstrated a clotting defect once (deficiency of the Hageman factor) and once found no signs of a clotting defect

A *fourth patient (family 51 no 4)* whose mother s brother suffers from haemophilia will be mentioned in this place One year old he was operated on for incarcerated hernia without bleeding abnormally From the age of 2 he

Fig

Haemophilic patients with no

Fam ly	P t t no	Ag	F rst m fest (y)	M ld w th y ars	H m th se	P ma f jo t d f rm t o	Bleed g n k	Bleed g muscles	G t o t i f haemo h g	Un ry t ct h mo h ges
24	4	18	2/12	+	–	–	+	–	–	–
29	[illegible]	49	10	29	+	–	–	+	–	+
56	4	22	2	14	–	–	–	–	–	–

Fig 51

A th	M th d	N mbe f pts. l ss f d	AHF d fi y	Ch tmas f t d f ency	Defi ncy f AHF pl Ch istm f t	PTA d fi y	H g m f t d fi ncy	C l t g tico gul t	No d m t bl cl tt g d fect
Beaumont Caen & Bernard (1954)	prothrombin consumption	35	-8) (80 %)	5 (14 %)				2 (6 %)	
Frick (1954)	clotting time prothrombin cons	55	45 (82 %)	6 (11)		4 (7 %)			
M C Rosenthal (1954)	clotting time prothrombin cons	40	32 (80 %)	6) (15 ,)		2 (5)			
R L Rosenthal (1954)	clotting time prothrombin cons	33	(74 %)	(15)		(11 ,)			
Soulier & Larrieu (1954)	clotting time	33	8) (85 %)	4 (12)	1 (3 %)				
Verstraete (1955)	thromboplastin generation test	4?	37 (88 %)	4 (10 %)	4 (2 %)				
Fantl & Sawers (1956)	clotting time thrombin generation	43	37 (86 %)	6 (14 %)					
Biggs & Macfarlane (1957)	thromboplastin generation test	164	139 (85)	-0 (12)		3 (? %)		2 (1)	
Sjølin	thrombin generation	148	7?) (49 %)	33) (-2 %)	6+18 +1 (16 %)	3 (2 %)	11 (7 %)	1 (0 7 %)	3 (2 %)

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thromboplastic activity in the plasma a fact which seems not to have been observed before The possibility cannot be excluded of course that this observation is due to the imperfection of our methods Another possibility is that the phenomenon is explainable by spontaneous variations in the thromboplastic activity (see family 8 no 6 and chapter X)

Three of the above patients belong to families with haemophilia defined as serum factor deficiency However a normal thromboplastic activity has also been found within the AHF defects Two such cases have been reported in the section dealing with the group named slight AHF deficiency

These were the patients nos 3 and 4 in family 88, who had primarily been classified in the group of PTA deficiency but who actually suffered from slight AHF deficiency their plasma being unable to normalise the thrombin generation in plasma presenting AHF deficiency (family 13 and family 59) None of these had haematological signs of haemophilia at the time of the investigation and they were fully fit

10 Comparison of the Danish Material with Other Comprehensive Materials

The table fig 51 gives a survey of the comprehensive haemophilic materials investigated in other countries compared with the Danish

It is stated in the previously published works that between 75 and 85 per cent of the haemophilic patients lack the antihaemophilic factor whereas no more than a scant half of the Danish patients lack the AHF alone Among these are even included 11 patients with slight AHF deficiency

As for the incidence of Christmas disease the statements range between 10 and 15 per cent In the author s material the corresponding figure is 22 per cent including six with slight defects Correction for these gives an incidence of about 18 per cent

Combined defects are mentioned in no more than two works These are stated to occur in 2–3 per cent whereas in the Danish series they constitute 16 per cent Among these are however included the defects classified primarily as 1) deficiency of the antihaemophilic factor plus freezing/serum factor and 2) a slight combined defect (patient no 1 family 77) After correction for these two groups there are still 4 per cent combined defects in the Danish series As mentioned previously there is reason to regard these two groups as mild combined defects

The incidence of PTA deficiency is somewhat lower in the Danish material than in most of the foreign This may be due to inclusion of other clotting defects (slight AHF deficiency slight Christmas factor deficiency mild combined defects) which makes the group too comprehensive The foreign materials also comprise women with PTA deficiency

Chapter IX

COMPARISON OF THE QUALITATIVE CLOTTING DEFECT WITH SYMPTOMATOLOGY AND WORKING CAPACITY

The table p 122 gives a survey of 1) the frequencies of the various bleeding phenomena in the different clotting defects and 2) the relation between the clotting defect and the working capacity of the patients aged 18 or more The patients in the group of Miscellaneous have not been included owing to their small number

1) *Frequencies of the Various Bleeding Phenomena*

Some reservation must be made with regard to these figures because the av erage age and the age distribution are not uniform for the patients with the different clotting defects The total number of abnormal bleeding phenomena will rise with increasing years in the individual patient On the other hand the different forms of bleeding occur the earliest where the clotting defects are the severest Furthermore some of the groups are so small that percentage figures are difficult to state Nevertheless the table gives a fairly good estimate of the frequencies of the various manifestations of the clotting defects

The severest haemorrhages (haemarthroses resulting in permanent joint de formities intramuscular haemorrhages) were seen to be the most frequent in patients with the combined defects They decreased in frequency in the order stated in the table The fact that haemarthroses were seen in only 83 per cent of the AHF plus Christmas group was due to one patient being no more than 5 years old at the time of investigation (1955) This patient had not yet had any unquestionable haemarthrosis

It seems as if the incidence of haemarthroses is a little lower among Christ mas patients than among patients with AHF deficiency The permanent changes resulting from haemarthroses were equally frequent in the two groups how ever

Bleedings in the skin were approximately equally frequent in all groups

Intramuscular bleedings occurred with almost the same frequency in the first four groups but were rare among the patients with the slight defects and

Seven per cent lacked the Hageman factor None of the previous surveys mention haemophilic patients with this mild clotting defect

Finally, there is the small group of apparently healthy patients in whose plasma no clotting defect was detectable This does not preclude however that they may have a haemophilic disease

The discrepancy may be due in the first instance to the following two facts

1 In such a small well organised country as Denmark the mild cases of haemophilia are easier to trace than in large countries There may be patients who are troubled so little by their disease that they only rarely give it a thought Furthermore in a small country it is more likely to be rumoured that somebody is working with some medical problem or other in this case haemophilia If a patient is admitted to hospital with an uncharacteristic haemorrhagic diathesis one is often informed by the colleagues involved

2 The other fact is the choice of testing methods

The investigations described show that the thrombin generation test is a satisfactory method in most cases It must be emphasized however that even by this method the mild cases of haemophilia may be classified in wrong groups as stated under the PTA and Hageman defects unless control tests are made with plasma samples having known clotting defects

The method has hitherto been employed to a limited extent only The table shows that the methods most frequently employed so far are the prothrombin consumption test and the thromboplastin generation test The author has no practical experience with regard to the former of these tests Using the latter test the author could not demonstrate the clotting defect in cases of slight Christmas disease The method may also fail in other mild defects (the Hageman trait) If further the clotting time is only slightly prolonged in such cases these are difficult to classify

This difficulty is probably due to the plasma of these patients being able to form (other) factors during the clotting which can compensate their own clotting defect If the patient s own serum is added to his own plasma as is done in the thromboplastin generation test the clotting defect of the plasma will not manifest itself

Platelet suspensions are also used in the thromboplastin generation test This likewise corrects a clotting defect (e g Hageman defect PTA deficiency)

In the cases of combined defects a slight Christmas defect may probably be missed by using the thromboplastin generation test

In the author s opinion the thrombin generation test makes it possible to work with a coagulation system that is more physiological than that on which the thromboplastin generation test is based as we here avoid introduction of partially unknown coagulation factors with serum platelet suspensions or lipoid

seemed not to occur at all in patients with the Hageman trait and PTA deficiency

Gastro-intestinal haemorrhage was noted in 50 per cent of the patients lacking the AHF and the Christmas factor whereas it was considerably less frequent in the remaining groups (9–19 per cent)

Haematuria was seen in over half of the patients with combined defects and in about half of the patients with slight Christmas factor deficiency

Gingival bleedings were a very common phenomenon Strangely enough it was most frequent in association with the mild defects This is possibly due to the fact that such patients relatively seldom have other forms of haemorrhage and therefore always remember the gingival bleedings which often are of a rather dramatic character The patients with severer clotting defects are troubled by so many other grave and painful haemorrhages that those from the gums are a relatively small problem to them

Bleedings in the central nervous system and the lower respiratory tract were very rare The author knows of two patients however who died from post traumatic epidural haematoma

Epistaxis occurred in the main in the stated order of frequency except that this sign was also very frequent among the patients with slight Christmas factor deficiency

The fact may be established on the basis of these investigations that the symptomatology is nearly the same in classical haemophilia and Christmas disease The previously published statements that Christmas disease generally is somewhat milder than classical haemophilia with regard to symptomatology could not be confirmed The most severely affected Christmas patients are just as poorly as the most severely affected patients with AHF deficiency Another thing is that treatment of Christmas patients is perhaps a little more gratifying

The question whether haemophilia grows milder in the course of years may be answered to the effect that the severer the clotting defect the smaller is the chance of abatement On the other hand it must be stressed that 25 per cent of the patients with classical haemophilia as well as of those with Christmas disease declared that the disease had grown milder That only one third of the patients with PTA deficiency admitted a decrease of the signs and symptoms must be accepted with some reservation as the group comprised only *three patients of whom two had always been troubled but little by their disease*

2) *Relation Between Clotting Defect and Working Capacity*

The investigations into this problem gave univocal results The working capacity was found to decrease with increasing clotting defect All the patients with AHF plus Christmas factor deficiency were almost totally disabled at least unfit for bodily work 20 per cent of the group lacking the AHF plus freezing/

Comparison of the qualitative clotting defect with symptomatology and working capacity

	AHF + Christmas factor	AHF + [illegible] factor	AHF	Christmas factor	Slight AHF deficiency	Slight Christmas factor deficiency	Hageman factor	PTA
Bleeding phenomena	*6 pts*	*18 pts*	*61 pts*	*27 pts*	*11 pts*	*6 pts*	*11 pts*	*3 pts*
Haemarthroses	5 (83%)[1]	18 (100%)	56 (92%)	21 (78%)	7 (50%)	3 (50%)	5 (45%)	1 (33%)
Permanent joint deformities	5 (83%)	15 (83%)	36 (59%)	15 (56%)	3 (42%)	1 (17%)	0	1 (33%)
Skin bleedings	6 (100%)	18 (100%)	56 (92%)	27 (100%)	11 (100%)	6 (100%)	10 (91%)	3 (100%)
Intramuscular bleedings	3 (50%)	6 (33%)	27 (44%)	11 (41%)	2 (18%)	1 (17%)	0	0
Gastro intestinal bleedings	3 (50%)	3 (17%)	7 (12%)	5 (19%)	1 (9%)	1 (17%)	1 (9%)	0
Bleedings from the urinary tract	4 (67%)	11 (61%)	27 (44%)	11 (41%)	2 (18%)	3 (50%)	1 (9%)	1 (33%)
Gingival bleedings	4 (67%)	14 (78%)	35 (57%)	18 (67%)	9 (82%)	5 (83%)	11 (100%)	3 (100%)
Bleedings in the central nervous system	0	0	1 (2%)	0	0	0	0	0
Epistaxis	4 (67%)	10 (56%)	26 (43%)	17 (63%)	5 (45%)	4 (67%)	5 (45%)	1 (33%)
Bleedings in the respiratory tract	0	0	0	3 (11%)	0	0	0	0
Milder with increasing years	0	4 (22%)	15 (25%)	7 (26%)	5 (45%)	3 (50%)	6 (55%)	1 (33%)
Patients > 18 years	*5 pts*	*10 pts*	*27 pts*	*17 pts*	*9 pts*	*3 pts*	*7 pts*	*2 pts*
Fully fit	0	2 (20%)	9 (33%)	8 (47%)	7 (64%)	3 (100%)	7 (100%)	2 (100%)
Partially fit	0	4 (40%)	9 (33%)	4 (24%)	2 (18%)	0	0	0
Unfit for working	5 (100%)	4 (40%)	9 (33%)	5 (29%)	0	0	0	0
Disablement benefit	5 (100%)	3 (30%)	9 (33%)	6 (35%)	0	0	0	0

[1] For comparison the approximate percentages were calculated and presented by the figures in brackets.

Chapter X

IS THE CLOTTING DEFECT CONSTANT IN HAEMOPHILIC PATIENTS?

Schloessmann as early as 1930 emphasized that the clotting time for blood from a haemophilic patient is always the same except for minor variations referable to the technique of testing

This theory was accepted by *Andreassen* who nevertheless using Burker s method found appreciable variations in the clotting time in the same patient It should be noted however that except in a single case repeated tests never gave a normal clotting time

Quick & Hussey (1952) held the same view They attributed changes in the bleeding tendency to other factors e g vascular The natures of such changes do not seem to have been investigated These writers examined a haemophiliac several times over a 6 year period and found clotting times (Lee White s method) ranging between 30 and 110 minutes the majority between 45 and 60 minutes The fairly great range of variation was referred to different tech niques of blood sampling among others and not to variations in the clotting defect On simultaneous prothrombin consumption test the clotting defect seemed to be constant

Hill & Speer (1955) have described two patients with combined haemo philia (AHF plus Christmas factor deficiency) whom they had previously classified in the group of pure AHF deficiency They both had haemorrhages at the time of investigation One of the patients seemed during a remission to have AHF deficiency alone whereas the other on subsequent investigation continued to have a double defect

According to the literature available there is probably reason for scepticism with regard to the postulate that the clotting defect in haemophiliacs is constant whereas the bleeding tendency may vary

Present Investigations

The author has submitted blood from a great number of haemophilic patients to two or more thrombin generation tests A few examples of these investiga tions will be given below Reference may be made also to the case reports at the end of the book

serum factor which is here regarded as a milder variant of the above group were fit i e able to support themselves and a family despite periods of severe illness

Perfectly fit were further 33 per cent of the AHF group 47 per cent of the Christmas group 88 per cent of the group of slight AHF deficiency 100 per cent of that of slight Christmas deficiency and likewise 100 per cent of the group lacking the Hageman factor and PTA

All the patients with severe combined defects receive disablement benefit In the three groups AHF plus freezing/serum factor AHF and Christmas factor about one third of the patients received disablement benefit In the remaining groups no patients were so disabled as to receive disablement benefit

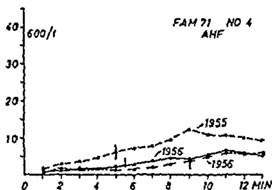


Fig 54

See legend to fig 57

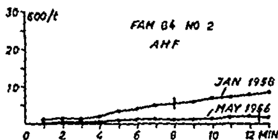


Fig 55

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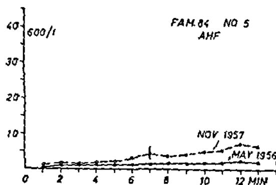


Fig 56

S e le end to fig 57

Classical Haemophilia (AHF Deficiency)

Figs 52–57 illustrate the results of analyses of blood from patients belonging to the families 69 71 and 84 all of which have members lacking the antihaemophilic factor In all the stated examples some variation is seen of the thromboplastic activity in the plasma In the samples from no 3 family 71, the thrombin generation was even nearly normal on a single test The vertical marks on the curves indicate the recalcification times The shortest recalcification time is seen to have been always accompanied by the best thrombin generation None of the samples were investigated within periods with a particularly pronounced bleeding tendency

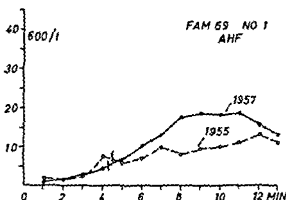


Fig 52

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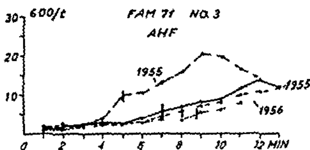


Fig 53

See legend to fig 57

Christmas Disease

Fig 59 shows the courses of the thrombin generation in a patient lacking the Christmas factor On investigation in 1957 no measurable amounts of thrombin were formed The recalcification time was scarcely 16 minutes One year pre viously measurable amounts of thrombin had been formed and the recalcifica tion time had been just over 8 minutes

The patient was in a quiescent period on both investigations

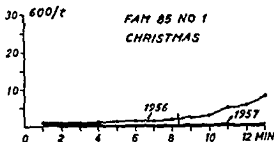


Fig 59

Courses of the thrombin generation in plasma from a patient lacking the Christmas factor inves tigated twice See also the legend to fig 57

Slight Christmas Factor Deficiency

Fig 60 illustrates the courses of the thrombin generation in a patient from family 8 whose blood was investigated twice at an interval of one year A considerable difference is seen in the rate of formation and the maximally obtained concentration The recalcification time was the shortest on the test where thrombin was generated at the fastest rate

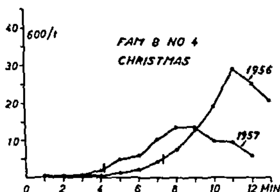


Fig 60

Course of the thrombin generation in plasma from a patient with slight Christmas factor defi ciency investigated twice See also the legend to fig 57

The patient had no bleeding phenomena at the time of the investigation

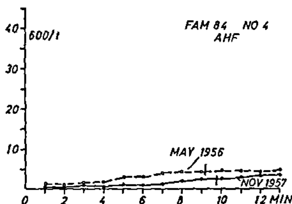


Fig 57

Figs 52–57 Courses of the thrombin generation in plasma from patients with AHF deficien y investigated several times The years of the investigations are stated on the curves The vertical marks on the curves indicate the recalcification times
Abscissa reaction time (T) in minutes
Ordinate Reciprocal value of the fibrinogen clotting time expressed as 600/t (t in seconds)

Slight AHF Deficiency

Fig 58 illustrates the course of the thrombin generation in a patient with slight AHF deficiency (family 92 no 2) Here too one test showed an almost normal thrombin generaton The shortest recalcification time was also found at this point of time The patient was in a period of remission on both investigations

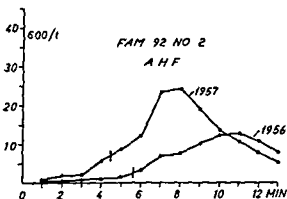


Fig 58

Courses of the thrombin generation in plasma from a patient with slight AHF deficiency investi gated twice See also the legend to fig 57

Figs 62 and 63 show the courses of the thrombin generation in repeatedly tested plasma from patients lacking the Hageman factor

In the first patient (family 51 no 4) the tests were made within the period 1955–1958 On the first two tests the thrombin generation and the recalcification time were normal On the last test the thrombin generation was delayed and the recalcification time slightly prolonged

In patient no 5 of the same family only one out of four tests revealed a clotting defect

In patient no 3 family 56 a clotting defect was found in 1955 but not in 1957 Here too parallelism was seen between the thrombin generation and the recalcification time

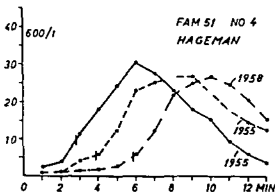


Fig 62

See legend to fig. 63

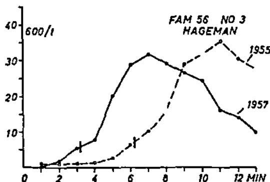


Fig 63

Figs 62–63 Courses of the thrombin generation in plasma from patients lacking the Hageman factor See also the legend to fig 57

Combined Defects

Fig 61 a illustrates the courses of the thrombin generation in blood from a patient lacking the AHF plus Christmas factor (family 1 no 4) Two tests were made at an interval of 2 months The thromboplastic activity was here uniformly poor on the two tests The recalcification times were 20 min and 16 min 30 sec

In this connection it should be mentioned that three patients (family 79 and family 103) classified as classical haemophiliacs on investigation in 1956 were referred to the group of double defects (deficiency of AHF plus Christmas factor) About 18 months later only AHF deficiency was demonstrable (See fig 61 b) The recalcification times were 18 min and 13 min 20 sec

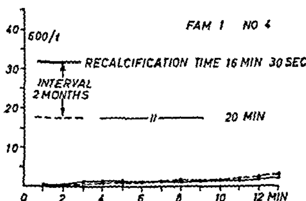


Fig 61 a

Courses of the thrombin generation in plasma from a patient with double defect AHF plus Christmas factor deficiency See also the legend to fig 57

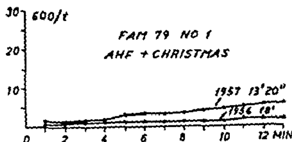


Fig 61 b

Courses of the thrombin generation in plasma from a patient who on the first test showed double defect (AHF plus Christmas factor deficiency) whereas on the second AHF deficiency alone The patient was in bed owing to haemarthroses on the first investigation See also the legend to fig 57

Figs 62 and 63 show the courses of the thrombin generation in repeatedly tested plasma from patients lacking the Hageman factor

In the first patient (family 51 no 4) the tests were made within the period 1955–1958 On the first two tests the thrombin generation and the recalcifica tion time were normal On the last test the thrombin generation was delayed and the recalcification time slightly prolonged

In patient no 5 of the same family only one out of four tests revealed a clotting defect

In patient no 3 family 56 a clotting defect was found in 1955 but not in 1957 Here too parallelism was seen between the thrombin generation and the recalcification time

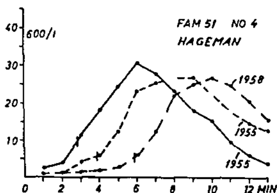


Fig 62

See legend to fig 63

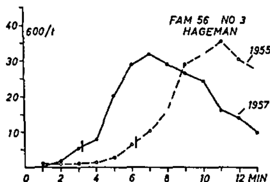


Fig 63

Figs 62–63 Courses of the thrombin generation in plasma from patients lacking the Hageman factor See also the legend to fig 57

Discussion

It is evident from what has been stated above that plasma from patients with the different forms of haemophilia may vary in thromboplastic activity In a few instances these variations were so considerable that plasma from the same patient showed pathological conditions and apparently quite normal conditions at different points of time

The first question suggesting itself is whether variations in the composition of the fibrinogen are responsible for the variations in the courses of the curves for the thrombin generation The varying recalcification times militate against this hypothesis In the great majority of the cases these varied parallel with the thrombin generation so that a poor thrombin generation was accompanied by a long recalcification time Thus a real variation seems to exist in the coagulability of the blood

Thrombocyte counts in no instance gave values low enough to account for the curves running the most level courses The poorest thrombin generation was even seen in association with the highest thrombocyte value in the same patient

The possibility must also be considered that tissue juice is admixed at the blood sampling This source of error was eliminated as far as possible by using only blood obtained by entering the vein cleanly The first few millilitres of blood were not used for the thrombin generation test in order to allow tissue thromboplastin if present to be washed away

That even small quantities of tissue thromboplastin influence the thrombin generation test has been shown by adding varying amounts of brain thromboplastin to the same haemophilic blood

Fig 64 curve A shows the course of the thrombin generation in haemophilic plasma (AHF deficiency) Curve B shows the course of the thrombin generation after addition of 0 2 ml of brain thromboplastin (Owren) diluted 1 10 to 1 ml of plasma In curve C the thromboplastin has been diluted 1 100, in curve D 1 1000

It is seen that very considerable amounts of brain thromboplastin are required to normalise the thromboplastic activity of the haemophilic plasma It seems unlikely that so much tissue thromboplastin should be admixed by a clean venous puncture that this might cause the alterations demonstrated in the coagulability of the haemophilic blood

According to the information available we may be justified in concluding that the clotting defect in the individual haemophilic patient may vary considerably

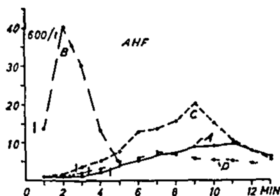


Fig 64

The thrombin generation in haemophilic plasma (family 84 no 2 AHF deficiency) after addition of varying amounts brain thromboplastin

Curve A no brain thromboplastin
- B 0 2 ml of brain thromboplastin diluted 1 10 to 1 ml of plasma
- C brain thromboplastin diluted 1 100
- D brain thromboplastin diluted 1 1000

Abscissa and ordinate see fig. 57

Chapter XI

THROMBOCYTE COUNTS IN PLASMA FROM NORMALS AND HAEMOPHILIACS

As stated previously it is universally agreed that the clotting defect in haemophilic blood is due to a defect of the thromboplastic system of the plasma To the author s knowledge a difference in number of platelets between haemophilic and normal plasma has never been demonstrated The number of platelets in haemophilic blood is supposed to be the same as in normal blood (*Schloessmann* 1930)

The present fairly large material was submitted to systematic counts of thrombocytes *in* haemophilic *as well as in control plasma samples*

The dot diagram fig 65 shows the distribution of the thrombocyte numbers in 311 plasma samples from normals divided in classes of 10000 By plotting these figures in a cumulated frequency diagram we find a normal distribution The dot diagram fig 66 shows the distribution of the thrombocyte numbers in plasma from 150 haemophilic patients divided in classes of 10000 These likewise show a normal distribution when plotted in a cumulated frequency diagram

The standard deviations in the two materials were calculated from the *formula*

$$S = h \sqrt{\frac{\text{sum of } ft^2}{n} - \left(\frac{\text{sum of } ft}{n}\right)^2} \quad (\textit{Moroney } 1954)$$

Where $h =$ the class interval (50000)

$$t = \frac{MP - m}{h}$$

$m =$ assumed mean 275000

$MP =$ the class mid mark

$n =$ the number of observations

$f =$ all observations in the class

In the *control material* the standard deviation was

$$S = 50000 \sqrt{\frac{1789}{311} - \left(\frac{181}{311}\right)^2} = 116335$$

$$\text{Standard error} = \frac{\textit{standard deviation}}{\sqrt{n}} = 6595$$

Mean $= 304100 \pm 6595$

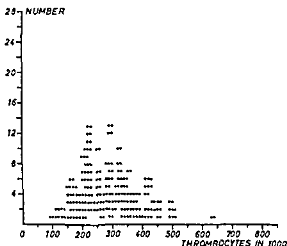


Fig 65

Dot diagram showing the distribution of the thrombocyte numbers in 311 normal plasma samples Ordinate number of normal samples Abscissa Thrombocytes in thousands per μl of plasma arranged in classes of 10000

In the *haemophilic material* the standard deviation was

$$S = 50000 \sqrt{\frac{2063}{150} - \left(\frac{261}{150}\right)^2} = 163752$$

Standard error = 13378

Mean = 362000 ± 13378

Difference of means

$$\bar{x}_p - \bar{x}_k = 362000 - 304100 = 57900$$

Standard error of difference

$$e_k^2 + e_p^2 = 6595^2 + 13378 = 14915$$

$$t = \frac{57900}{14915} = 3\ 88$$

p is about 0 1 / which means that the difference between the two means is significant

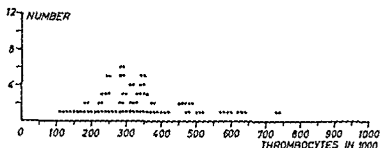


Fig 66

Dot diagram showing the distribution of the thrombocyte numbers in plasma samples from 150 haemophilic patients Ordinate number of plasma samples Abscissa thrombocytes in thousands per µl of plasma arranged in classes of 10000

By way of comparison the results of thrombocyte counts in previous Danish investigations will be stated below (*Heinild* 1942)

Oluf Thomsen	200000–413000
H C Gram	300000–550000
Kuhnel	255000–407000
Heinild	145000–370000

The present results agree fairly closely with those rendered above Heinild s values are the lowest with means for men and women of 229000 (standard error 42000) and 260000 (standard error 55000) respectively

Discussion

The author s thrombocyte counts gave a small but significant majority in the haemophilic plasma This is possibly to be regarded as an attempt by Nature to improve the impaired thromboplastic activity in the haemophilic plasma by increasing the contribution of the platelets towards the plasma thromboplastin

In two haemophiliacs the author obtained very low figures

In one of these (no 6 family 31) 17000 platelets were counted per µl of plasma A subsequent count gave 630000

In the other patient (no 1 family 67) the first count gave 92000 per µl of plasma and the second 788000

None of the two patients had petecchial haemorrhages at the time of thrombopenia

Chapter XII

TREATMENT

Though the treatment of haemophilia is beyond the scope of this work it is a problem of such clinical interest that it will be briefly discussed It must be emphasized immediately however that no cure has been found as yet against haemophilia

AHF Deficiency

The symptomatologic treatment of haemophilia mav be divided in local and general treatment

Local Treatment

The principle of the local treatment is that of placing a coagulant on an adsorbable substance in direct touch with the bleeding surfaces

As coagulants can be used Russell s viper venom (*Macfarlane & Barnett* 1934) or human or animal thrombin (*Lowner MacDonald Finland & Taylor* 1941 *Adams & Taylor* 1943) impregnated in human fibrin fibrin foam gelatin sponge calcium alginate or oxidised cellulose gauze which can all be left in place being absorbable (*Macfarlane & Biggs* 1955 *Biggs & Macfarlane* 1957) To ensure that the coagulant reaches the small bleeding vessels all clots will have to be removed A steady pressure must then be exerted for 5 to 10 minutes and thereafter slackened slowly If the clot produced dis appears by proteolysis or is washed away by renewed haemorrhage most often after a few hours the procedure will have to be repeated

Lewis Tagnon Davidson Minot & Taylor (1946) used as local haemostatics thrombin from rabbit plasma and pseudoglobulin from human bovine and pig s plasma

It is very important to avoid intervention which devitalises the tissue such as violent compression cauterization or burning As soon as the devitalised tissue is shed off renewed haemorrhage will set in from a larger surface The wound should be immobilised if possible

Dental Extraction

Haemorrhages following dental extraction being such a frequent phenomenon in haemophiliacs irrespective of the degree of the disease there is reason to enter further on the problem of dental extractions in these patients

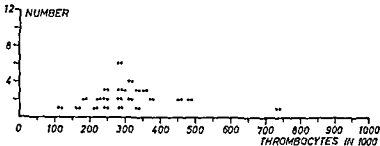


Fig 66

Dot diagram showing the distribution of the thrombocyte numbers in plasma samples from 150 haemophilic patients Ordinate number of plasma samples Abscissa thrombocytes in thousands per µl of plasma arranged in classes of 10000

By way of comparison the results of thrombocyte counts in previous Danish investigations will be stated below (*Heimild* 1942)

Oluf Thomsen	200000–413000
H C Gram	300000–550000
Kuhnel	255000–407000
Heimild	145000–370000

The present results agree fairly closely with those rendered above Heimild s values are the lowest with means for men and women of 229000 (standard error 42000) and 260000 (standard error 55000) respectively

Discussion

The author s thrombocyte counts gave a small but significant majority in the haemophilic plasma This is possibly to be regarded as an attempt by Nature to improve the impaired thromboplastic activity in the haemophilic plasma by increasing the contribution of the platelets towards the plasma thromboplastin

In two haemophiliacs the author obtained very low figures

In one of these (no 6 family 31) 17000 platelets were counted per µl of plasma A subsequent count gave 630000

In the other patient (no 1 family 67) the first count gave 92000 per µl of plasma and the second 788000

None of the two patients had petecchial haemorrhages at the time of thrombopenia

moribund owing to the loss of blood He then had 5½ ounces of blood transfused from a woman without preceding typing or stabilisation of the blood One hour after the transfusion the boy sat up in bed and haemostasis had been obtained No complications of compatibility occurred

Weil (1905) noticed a fall of the clotting time in a haemophiliac after intravenous injection of small amounts of human or bovine serum (Christmas patient?) The correcting influence of cell and platelet free plasma on the clotting of haemophilic blood was first detected by *Feissly* (1924) The clotting promoting principle the antihaemophilic factor first demonstrated by *Addis* (1911) was referred to the globulin fraction of the plasma (*Patek & Taylor* 1937)

Intravenous injection of milligram doses of the globulin fraction reduced the clotting time in 15 out of 16 haemophiliacs (*Minot Davidson Lewis Tagnon & Taylor* 1945) but the clotting promoting activity was preserved for no more than 8–12 hours after the injection (*Lewis Tagnon Davidson Minot & Taylor* 1946)

It was previously believed that transfusion of such small amounts of plasma as 50 ml would suffice to compensate the clotting defect in haemophilia this being able to normalise the clotting time Now we know that a normal clotting time does not preclude a defective thromboplastic activity of the blood (*Mershey* 1950 *Quick & Hussey* 1952 *Graham McLendon & Brinkhous* 1953 *Macfarlane & Biggs* 1955)

Brinkhous Langdell Penick Graham & Wagner (1954) and *Biggs & Macfarlane* (1957) state that the blood concentration of AHF must be not less than 30 per cent of the normal to give normal haemostasis and prevent bleeding from minor lesions For larger injuries and surgical interventions a concentration of 50 per cent is required Clotting time determinations and prothrombin consumption tests are thus of little value as a guidance to transfusion treatment

To raise the AHF concentration from 0 to 30 per cent in an average adult person would require a plasma transfusion of about 1½ litres (*Biggs* 1957) provided no blood was lost at the same time It is therefore very difficult to maintain such high concentrations by transfusions *Langdell Wagner & Brinkhous* (1955) in transfusion experiments never obtained AHF concentrations exceeding 20–25 per cent However they obtained a prompt haemostatic effect in a bleeding haemophilic patient at an AHF concentration as low as 6–14 per cent The same writers have shown that the concentration of transfused AHF falls by 50 per cent in the course of 2–3 hours both in haemophiliacs and in normals To maintain an AHF concentration of 5–10 per cent of the normal blood or plasma must be given every 2–4 hours Even if AHF concentrates are given the plasma concentration will be halved within 2 hours (*Langdell Wagner & Brinkhous* 1955)

It is most often stated that about half of the AHF activity in plasma stored at + 4° C is lost in the course of 10–14 days (*Pitney & Dacie* 1955 *Brink*

Unfortunately, the fear of these haemorrhages has often kept haemophiliacs from even the simplest dental prophylaxis Consequently, by the time a dentist is consulted the teeth of these patients are often too carious or infected to be saved by conservative treatment and therefore have to be extracted

Haemophiliacs should always have dental extraction performed in hospital where transfusions can be given immediately if required General anaesthesia is preferable to local anaesthesia The latter may cause large soft tissue haematomas which may spread to the neck where they may give rise to asphyxia Unless transfusion has been given prophylactically no more than two teeth should be extracted at a time (*Macfarlane & Biggs* 1955) After the tooth has been removed as atraumatically as possible the socket is plugged with fibrin foam moistened with thrombin or Russell viper venom and digital pressure is exerted for 5 to 10 minutes The haemostatic used can now be kept in place by a small splint It will most often be necessary to repeat the procedure after a few hours

Here as in other forms of local treatment care should be taken to avoid any rough manipulation Specially suturing of the gingival edges is advised against because this may result in diffuse blood infiltration of the tissues involving a risk of compression of the cervical organs

Deep Tissue Haemorrhages

Quick (1957) recommends the use of a moderate local pressure in cases of deep tissue haemorrhages e g with adhesive tape placed parallel to the blood stream to increase the interstitial pressure and thus inhibit the diffuse bleeding into the tissue Further he recommends a cold stimulus to bring about a reflectory contraction of the blood vessels Immediately after an injury it is advisable to place an ice bag on the skin above the site of the injury and keep the area cold for 24 hours

Intra abdominal haemorrhages extra as well as intraperitoneal may simulate acute abdomen but must of course be treated conservatively More particularly retroperitoneal haemorrhages may simulate acute appendicitis

Extravasations of blood into the soft tissues of the oral cavity and throat may as stated be extremely dangerous owing to the risk of asphyxia Dyspnoea must be prevented by early intubation or if the soft tissue swelling renders this impossible tracheotomy rendered safe as far as possible by transfusions

General Treatment

The general treatment of haemophilia aims at restoring the coagulability of the blood Our only chance to-day of doing so is by way of transfusing whole blood or plasma or injecting the individual plasma thromboplastin factors

Lane (1840) was presumably the first to stop a bleeding in a haemophilic patient by blood transfusion The patient was an 11 year old boy who had been operated on for a squint according to Dieffenbach s method He began to bleed a few hours after the operation and within a few days became

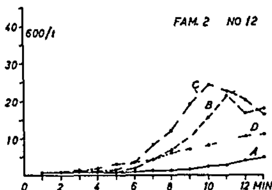


Fig 67

Courses of the thrombin generation in plasma from a patient with AHF deficiency after transfusion of 1 litre of blood Abscissa and ordinate as in fig 57 See also the text

Fig 67 curve A shows the thrombin generation in plasma from patient no 12 family 2 Recalcification time 13 min 2 hours after the patient had received 1 litre of citrated blood corresponding to about one fifth of his blood volume the course of the thrombin generation was as shown in curve B Appreciable amounts of thrombin were now formed but only after a lag period of 6 minutes The recalcification time was 7 min 30 sec Twelve hours after the transfusion the thrombin generation was found to be almost unchanged (curve C) and the recalcification time was 6 min 25 sec Seventeen and a half hours after the transfusion the thrombin generation (curve D) was almost as poor as before the transfusion but the recalcification time was still somewhat shorter 7 min 30 sec

Fig 68 shows the thrombin generation in an 8 year old boy with AHF deficiency (family 71 no 3) Curve A represents the thrombin generation

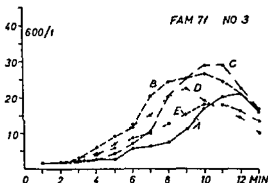


Fig 68

Courses of the thrombin generation in plasma from a patient with AHF deficiency after transfusion of fresh blood Ab cissa and ordinate as in fig 57 See also the text

hous Penick Langdell Wagner & Graham 1956) Spaet & Kinsell (1953) even found half of the AHF activity to have been lost after 24 hours and that at refrigerator temperature

Penick & Brinkhous (1956) investigated the AHF stability in plasma under different conditions of storage and found it to increase with falling temperatures At refrigerator temperature most of the AHF activity was destroyed between the first and the third week of storage, after which period the remainder seemed to be more stable Freezing reduced the antihaemophilic activity by 50 per cent in the course of 1 month after which the loss of activity wa very small At 56° C the AHF was inactivated very quickly whereas it was very stable in freeze dried plasma The AHF seemed to be less stable in human than in animal plasma Traces of thrombin and fibrinolysin also reduced the stability of the AHF

The stability is greater in citrated than in oxalated plasma (*Spaet & Garner* 1955 *Brinkhous Penick Langdell Wagner & Graham* 1956)

The instability in oxalated human plasma is due to the presence of a heat stable dialysable inactivator which when added to bovine plasma destroys the AHF of this (*Spaet & Garner* 1955)

Serum contains no AHF (*Aggeler White Glendening Page Leake & Bates* 1952 *Biggs Douglas Macfarlane Dacie Pitney Merskey & O Brien* 1952)

The fall noticed by *Weil* (1905) in the clotting time of haemophilic blood after injection of serum was presumably due to the patient having Christmas disease However *M C Rosenthal* (1954) found in a few instances that serum could give a normal clotting time and a normal recalcification time in haemophiliacs (AHF deficiency) but not a normal prothrombin consumption (cf beides p 81) Adsorption of serum with barium sulphate has been found to correct the clotting defect in a few patients with AHF deficiency (*Sjölin 1957*)

Summarising we may say that blood withdrawn carefully with sodium cit rate as stabiliser is an excellent source of AHF when used within a few days of withdrawal and stored at + 4° C or lower temperatures In the majority of cases it will suffice to transfuse fresh plasma in an amount corresponding to 20 per cent of the patient s blood volume (*Biggs & Macfarlane* 1957) and will continue to give this amount distributed over four transfusions per day until 24 hours after haemostasis has been obtained (*Stefanini & Dameshek* 1955) One must however be prepared to use large quantities of blood and plasma

Present Investigations

The author has not made quantitative AHF determinations (*Biggs Eveling & Richards* 1955) but has in a few cases had occasion to follow the thromboplastic activity of the plasma by means of the thrombin generation test in haemophiliacs after transfusion

The recalcification time had fallen to 7 min 45 sec Thirty six hours after the transfusion the thrombin generation was the same (curve C) as after 6 hours or perhaps slightly better The recalcification time was 6 min 30 sec Seventy two hours after the transfusion the thrombin generation was decreasing (curve D) and the recalcification time was rising 8 min 35 sec

It is evident from the curves that one litre of blood was quite insufficient to normalise the thrombin generation The patient also had melaena before during and after the transfusion which had to be repeated twice The fact that some improvement of the thrombin generation persisted for at least 72 hours was presumably due to introduction of Christmas factor which is stated to remain in the blood circulation for up to 2 or 3 weeks (*Beaumont Caen & Bernard* 1954)

The author directed the transfusion treatment of a 42 year-old patient with AHF deficiency (family 10) who was to be operated on for a large inguino-scrotal hernia Immediately before the operation venesection was performed and 500 ml of blood were allowed to escape Then 1000 ml of plasma were transfused As the thrombin generation test now showed a normal coagulation process the patient was operated on Topostasin (Roche) was used locally During the operation another 500 ml of plasma were transfused at a slow rate The next 10 days fresh plasma was given about 1 litre daily distributed over 2–3 transfusions Further venesection was performed twice with escape of 500 ml of blood The clotting status was examined daily by the thrombin generation test and found to remain normal

The patient did not bleed postoperatively The wound healed up by first intention and he was discharged in good health 12 days after the operation after having received about 12500 ml of plasma

The uneventful course was due in part to the fact that the patient probably did not completely lack the AHF This facilitated maintenance of a high AHF concentration in the blood It is essential that the treatment is started before the operation to prevent haemorrhage from beginning at all Otherwise it is difficult to maintain a high AHF concentration Further it is important that the disease is in a quiescent phase with no spontaneous bleeding tendency

Disadvantages of Transfusion

One of the most important difficulties involved by transfusion treatment of classical haemophilia (AHF deficiency) is that of procuring and administering the large amounts of blood and plasma that may be required to obtain hae mostasis

A complication which is rare but serious to the individual patient is the development of inhibitors caused by transfusions *Munro & Jones* (1943) fol lowed through several years a haemophilic patient who received trans fusions The response to these was excellent at first but subsequently the clinical

before the transfusion The recalcification time was 6 min 30 sec After transfusion of 150 ml of fresh blood there was found some improvement of the thrombin generation (curve B) and the recalcification time was 4 min 30 sec Four hours after the transfusion the thrombin generation was practically unchanged (curve C) The recalcification time was 5 min 50 sec Twenty one hours after the transfusion the thrombin generation was nearly normal (curve D) and the recalcification time was prolonged 5 min 30 sec 45 hours after the transfusion the thrombin generation was as before this (curve E) and the recalcification time was 5 min 30 sec

In the former of these two patients the amount of blood transfused was unable to normalise the recalcification time and the thrombin generation The thrombin generation was as poor as before the transfusion already before the elapse of 24 hours The patient had melaena before during and after the transfusion

In the latter instance where the spontaneous thromboplastic activity of the plasma was somewhat better than in the former the recalcification time was within the normal range immediately after the transfusion Twenty one hours after the transfusion the thrombin generation was nearly normal The effect on the coagulation system was totally lost after about 48 hours The transfused amonut of blood was too small in both cases

The author has had occasion to follow the thrombin generation in a 46 year old patient (family I no 4) with AHF plus Christmas factor deficiency Fig 69 curve A shows the thrombin generation before the transfusion Practically no thrombin was formed and the recalcification time was 16 minutes Six hours after transfusion of 1 litre of citrated blood the course of the thrombin generation was as shown in curve B Considerable amounts of thrombin were formed after a lag period of 6–7 minutes but the course of the curve was not normal

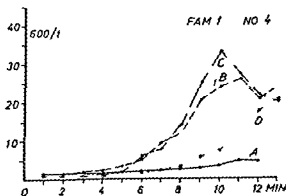


Fig 69

Courses of the thrombin generation in plasma from a patient with AHF plus Christmas factor deficiency after transfusion of 1 litre of blood Abscissa and ordinate as in fig 57 See also the text

100 ml had an effect corresponding to that of 1000 ml of plasma After having injected this concentrate they performed minor operations without abnormal haemorrhages The only complication was one of urticaria in a few patients *Blomback & Nilsson* (1957) administered large doses (1–3 g) of human AHF to haemophilic patients and obtained a good clinical effect Two days after ehe injection the AHF concentration had fallen by no more than about 50 per cent

Like transfusion treatment treatment with human AHF preparations may cause development of anticoagulants and phases refractory to treatment (*Pohle & Taylor* 1938 *Craddock & Lawrence* 1947 *Creveld Hoorweg & Paulssen* 1951 *Frommeyer Epstein & Taylor* 1950)

An evaluation of the AHF preparations is as yet difficult partly owing to variations in their potency (*Bidwell* 1955) and partly because of the spontaneous variations in the state of health of haemophiliacs There is hardly any doubt however that the production of animal AHF preparations represents a large step forward in the treatment of haemophilia and that these preparations have saved the lives of several patients (*Macfarlane et al* 1957) Nevertheless owing to the stated elements of risk they should be used only in emergencies The animal preparations cannot be used again later for the same patient An ideal substitution therapy in haemophilia has not yet been found

Christmas Disease

The local treatment of Christmas disease does not differ from that of classical haemophilia

The literature gives only relatively few details concerning the general treatment of Christmas disease

As stated previously the Christmas factor is rather stable and present in both plasma and serum Apparently it is not consumed during the clotting In agreement with this various workers have stated that transfused Christmas factor has effect on the recipient for 2–3 weeks (*Beaumont Caen & Bernard* 1954 *Aggeler Spaet & Emery* 1954)

Stefanini & Dameshek (1955) found transfused Christmas factor in the blood for up to 6 days after the transfusion

The antihaemophilic factor has no influence on the clotting time in the blood of Christmas patients (*Aggeler White Glendening Page Leake & Bates* 1952 *Creveld & Paulssen* 1953)

Present Investigations

The author has had occasion to follow the thrombin generation after transfusion in a 9 year old boy with severe Christmas disease (family 4 no 10)

Fig 70 curve A shows the thrombin generation in the patient's plasma before the transfusion The recalcification time was 10 min 30 sec Imme

state deteriorated after the transfusions while the clotting time lengthened Similar observations were mad by *Frommeyer Epstein & Taylor* (1950) who among 22 patients with classical haemophilia found five whose disease had become refractory to therapy Hence, there is reason to warn against pro phylactic transfusions, which anyhow give only short improvement of the coagulability of the blood, and reserve the transfusions for the severe acute haemorrhages If one is in the situation of having to control the stage re fractory to therapy, one must transfuse very large amounts of whole blood and/ or plasma to neutralise the anticoagulants and precipitins formed Incidentally, these tend to disappear in the course of months or years but are quickly re formed (*Frommeyer Epstein & Taylor* 1950)

Among the possible complications of transfusion treatment of haemophilia there is also the risk of transmitting hepatitis Finally in a single case thrombo cytopenia has been seen to develop following transfusions because of in compatibility of the platelet types (*Stefanini & Dameshek* 1955)

Treatment With the Antihaemophilic Factor

It has been stated previously that the human AHF is very unstable compared with the animal AHF (*Spaet & Kinsell* 1953 *Spaet & Garner* 1955 *Penick & Brinkhous* 1956) presumably due to presence of an inactivator in human plasma (*Spaet & Garner* 1955) Within recent years great activity has there fore been displayed especially by British workers to isolate the antihaemo philic factor from animal plasma

Spaet & Kinsell (1953) prepared from bovine plasma an AHF having an activity per milligram protein which was 70 times higher than that of the human AHF *Lorand & Laki* (1954) indicated a method of preparing pro thrombin activator (AHF?) from human canine and bovine plasma *Bidwell* (1955) prepared a very active AHF preparation from bovine plasma as well as plasma from pigs and sheep The bovine AHF has been used clinically in association with surgical interventions with a favourable result (*Macfarlane Biggs & Bidwell* 1954 *Macfarlane Mallam Witts Bidwell Biggs Fraenkel Honey & Taylor* 1957)

Unfortunately both bovine and ovine AHF have been found to agglutinate the human platelets whereas AHF from pigs do not (*Bidwell* 1955) The animal AHF having an antigenic action its use is limited to 10–14 days In cases of a short need for AHF this disadvantage can be evaded by changing from one animal AHF preparation to another Further the animal AHF pre parations may give rise to anaphylactic reactions in sensitised patients (*Biggs & Macfarlane* 1957)

Macfarlane et al (1957) saw no development of anticoagulants in 13 pa tients treated with animal AHF preparations

Kekwick & *Wolf* (1957) prepared a concentrate of human AHF of which

hours after transfusion of 1/2 litre of citrated blood the thrombin generation was found to have improved as shown in curve B and the recalcification time had fallen to 5 min 30 sec Twenty one hours after the transfusion the thrombin generation was further improved (curve C) and the recalcification time was 4 min 30 sec As late as 72 hours after the transfusion there was still some thrombin generation (curve D) but the recalcification time was increasing in length 6 min 30 sec Fifteen days after the transfusion no response was traceable The recalcification time was 11 min 30 sec (curve E)

The transfused amount of blood had been too small to completely normalise the thromboplastic activity of the plasma Seventy two hours after the transfusion some improvement of the thrombin generation was still traceable but the prolonged effect mentioned in the literature was not demonstrable in this case either

Fig 72 shows the thrombin generation in plasma from a third patient with Christmas disease (family 22 no 19) 16 hours after transfusion of 1/ litre of fresh blood (curve A) The thrombin generation was normal Forty eight hours after the transfusion the conditions were as shown in curve B The recalcification time was 8 min and the thrombin generation poor In this case a perfectly normal thrombin generation was obtained after transfusion of 1/2 litre of blood but the response was very transitory

Compared with transfusion treatment in cases of AHF deficiency it seems as if a normal thromboplastic activity can be obtained in Christmas patients with somewhat smaller amounts of blood or plasma which need not be freshly drawn from a donor but a prolonged effect has not been demonstrable Finally it may just be mentioned that serum can be used in the treatment of Christmas disease

For the present transfusion treatment of Christmas disease should be given according to the same scheme as that of classical haemophilia

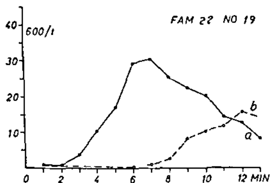


Fig 72

Courses of the thrombin generation in plasma from a patient with Christmas disease after transfusion of ½ litre of blood Abscissa and ordinate as in fig 57 See also the text

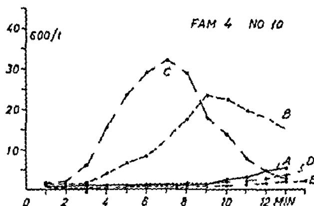


Fig 70

Courses of the thrombin generation in plasma from a patient with Christmas disease after transfusion of 300 ml of fresh blood Abscissa and ordinate as in fig 57 See also the text

diately after the transfusion (300 ml of fresh blood) the thrombin generation was somewhat improved (curve B) and the recalcification time was 5 min Six hours after the transfusion the thrombin generation was normal (curve C) and the recalcification time 3 min 30 sec Twenty four and 48 hours after the trans fusion the thrombin generation was as poor as before the transfusion (curves D and E) The recalcification times were 14 and 12 minutes respectively

Though the thromboplastic activity of the plasma became normal in this case the effect of the transfusion was of very short duration by no means corresponding with what has been stated in the literature

Fig 71 curve A shows the thrombin generation in a 17 year old youth with severe Christmas disease (family 14 no 10) No thrombin whatever was formed in the course of 21 minutes The recalcification time was 11 min Four

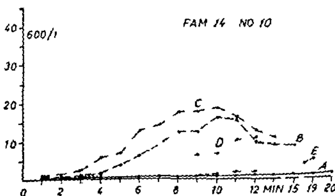


Fig 71

Courses of the thrombin generation in plasma from a patient with Christmas disease after transfusion of ½ litre of blood Abscissa and ordinate as in fig 57 See also the text

cerned The PTA factor is very stable its activity being present in plasma and serum stored at –10 to –20° C for 2 years Sterile plasma stored at room temperature for 4 months had a normal PTA activity (*Rosenthal* 1955)

The clotting time for blood from patients with PTA deficiency remains normal for 7–10 days after transfusion of serum or stored plasma (*Stefanini* 1954)

Rosenthal Dreskin & *Rosenthal* (1955) found a normal clotting time in a patient with PTA deficiency for 7 days after transfusion of 450 ml of 7 days old blood bank plasma This single transfusion sufficed for performing a hernioplastic operation without complications The author has not had occasion to follow the thromboplastic activity of the plasma after transfusion to patients with PTA deficiency

Treatment of the Hageman Trait

As stated previously only a few of the cases of the Hageman trait reported so far have displayed clinical signs of a haemorrhagic diathesis Many of these patients have even been operated on without haemorrhagic complications (*Rat noff* & *Colopy* 1955 *Frick* & *Hagen* 1956 *Ramot Singer Heller* & *Zimmer mann* 1956 *Sjølin* 1957 b) The Hageman factor is present in serum as well as plasma (*Ratnoff* & *Colopy* 1955) These authors found that transfusion of 250 ml of citrated plasma to a Hageman patient effected a fall of the clotting time from 60 to 12 minutes Eight hours after the transfusion the clotting time was however already 65 minutes *Ramot Singer Heller* & *Zimmerman* (1956) obtained a similar effect with as little as 50 ml of blood stored for 20 days *Larrieu Soulier* & *Culot* (1957) corrected transitorily the clotting defect in an adult Hageman patient with 150 ml of plasma

Present Investigations

The author has had occasion to follow the thrombin generation in a patient with a haemorrhagic diathesis due to deficiency of the Hageman factor (family 112 no 2) The most interesting fact about this patient was that he began to bleed from a tooth socket at a time when the thromboplastic activity of the plasma was normal (see p 340) Haemostasis was obtained only by daily transfusions the following 4 days

Patients having the Hageman trait with an accompanying haemorrhagic diathesis should when submitted to a surgical intervention be treated pro phylactically by plasma transfusions about ½ litre to be continued daily until the wound has healed up

Combined Defects

Combined clotting defects should be treated on the same principles as classical haemophilia (see p 140)

Treatment with Christmas Factor

American workers have already published some papers on isolation of the Christmas factor (*Aggeler White Glendening Page Leake & Bates* 1952 *White Aggeler & Emery* 1953 *White Aggeler & Glendening* 1953 *Aggeler Spaet & Emery* 1954)

White Aggeler & Emery (1953) found that intravenous injection of fraction IV of the plasma proteins shortened the clotting time for up to 7 days and improved thr prothrombin consumption for up to 4 days However the improved prothrombin consumption began to decline again 3–4 hours after the injection

Schilling (not published) using Aggeler s method produced a preparation of the Christmas factor which the author has had occasion to test *in vitro* on plasma from the first two of the above mentioned Christmas patients Fig

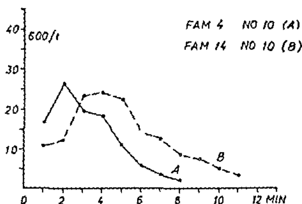


Fig 73

The thrombin generation in plasma from the patients fig 70 (curve A) and fig 71 (curve B) after addition of isolated Christmas factor *in vitro* Abscissa and ordinate as in fig 57

73 curves A and B show the thrombin generation in plasma from the two patients described in the preceding section (figs 70 and 71) after addition of this Christmas preparation In both cases a striking effect was seen with rapid formation and inactivation of the thrombin The recalcification times were 30 and 50 seconds respectively Thus there seems to be good reason for continuing to aim at isolating the Christmas factor from human blood the more so because the raw materials of this factor stored blood and serum are relatively easily available

Treatment of PTA Deficiency

Our experience with regard to treating patients with PTA deficiency is slight However, the disease generally runs such a mild course that the problem of treatment is smaller than where the two above forms of haemophilia are con

Chapter XIII

THE FEMALE MEMBERS OF THE HAEMOPHILIC FAMILIES

Do Female Haemophiliacs Exist?

By true haemophilia in females we understand a condition in which the gene for haemophilia is present in the homozygous form in the female organism Such can only be possible in the offspring of a haemophiliac and a transmitter of haemophilia

Theoretically haemophilia in a female may be conceived to arise by mutation of the healthy gene in a heterozygote or by mutation of both genes in a healthy woman into haemophilic genes

According to *Andreassen* (1943) no unequivocal cases of true haemophilia in females had been observed up to the time when his book was written

In 1951 *Pinniger & Franks* described a woman aged 21 with a haemorrhagic diathesis manifesting itself by prolonged haemorrhages from cuts and by haemarthroses

The patient s father as well as her maternal grandfather were haemophiliacs so that she had a 50 per cent chance of having the haemophilic gene in the homozygous form

Israels Lempert & Gilbertson (1951) mentioned a patient whose father was a haemophiliac and whose mother had a haemophilic brother The patient had a prolonged clotting time and a raised serum prothrombin level She bore an apparently healthy daughter Uterine haemorrhage occurred in the puerperium so that hysterectomy had to be done If this patient s mother was a transmitter the patient had 50 per cent chance of being homozygous for haemophilia

Merskey (1951 b) gives an account of a haemophilic family in which a haemophiliac had married his cousin They had two haemophilic boys four haemophilic girls three healthy girls and one healthy boy Three of the haemophilic girls were married and all their sons were haemophilic The three haemophilic girls thus seem to have been homozygotes Merskey found a reduced capillary resistance in two of the haemophilic girls so the possibility that they suffered from a different haemorrhagic diathesis cannot be definitely excluded

Brinkhous Graham Penick & Langdell (1951) by mating heterozygous

Treatment of Patients Having Inhibitors in the Blood

Patients with inhibitors in the blood are in a very bad way when bleeding as they require transfusion of very large amounts of blood (1½–2 litres) to obtain temporary improvement of the coagulability (*Frommeyer et al* 1950) an improvement which is often followed by a rise of the inhibitor action Even such large amounts of blood cannot always correct the clotting defect (cf *Hougie* 1955 b) The antithromboplastic action may decrease appreciably during prolonged interruption of the transfusion treatment (*Munro & Jones* 1943 *Frommeyer Epstein & Taylor* 1950)

Several investigations have been made into the influences of ACTH and cortisone on the anticoagulants in haemophilia (*Verstraete* 1955, *S neer Mord Hyman & Levy* 1950) These drugs have never been seen to cause either clinical or haematological improvement Neither are protamine sulphate toluidine blue or testosterone able to improve these conditions (*Verstraete* 1955)

As to all appearances the development of an anticoagulant is related to the transfusion treatment we must strongly warn against transfusions as a routine roborant treatment in haemophilia Bleeding episodes in patients with inhibitors may necessitate exchange transfusion

Skold (1952) found a just significantly longer clotting time in transmitters than in normals *Pianta* (1953) observed a prolonged clotting time in two out of nine transmitters and *Sjølin* (1954) in 22 out of 27

Merskey & Macfarlane (1951) found that measurement of the clotting time as well as determination of the prothrombin consumption are unfit for diagnosing heterozygosity

Within recent years transmitters have been investigated by the prothrombin consumption test *Jurgens & Ferlin* (1950) found a reduced conversion of prothrombin in transmitters though not so pronounced as in the haemophiliacs

Douglas & Cook (1957) observed a reduced thromboplastin formation and prothrombin consumption in two sisters who were daughters of a haemophiliac There were other male haemophiliacs (AHF deficiency) in the family

Brinkhous Graham Penick & Langdell (1951) found the prothrombin consumption to be reduced in heterozygous dogs

In a family with mild classical haemophilia *Koller Krusi & Luchsinger* (1950) demonstrated a reduced prothrombin consumption in the transmitters

The same was observed by *Biggs Douglas Macfarlane Dacie Pitney Merskey & O Brien* (1952) in the mother of a Christmas patient

In the Swiss Tenna family in which the patients have Christmas disease *Huser & Moor Jankowski* (1957) saw normal conditions of coagulation in 11 unquestionable transmitters and 32 possible transmitters

Graham McLendon & Brinkhous (1953) found an abnormally low AHF concentration in plasma from transmitters in a haemophilic family with mild manifestations of the disease

Thus most of the literature confirms that some clotting defect is demonstrable in several transmitters of haemophilia No quite satisfactory method is available as yet for detecting heterozygosity in the individual female

Present Investigation

The author has not included in his investigations systematic examinations of transmitters of haemophilia

However 18 women having haemophilic sons have been classified on the basis of the clotting defect in these The clotting times in the stated women have been published previously (*Sjølin* 1954) but the haemophilic patients had not then been classified on the basis of their clotting defects

This classification is shown in the table fig 74

Nine out of ten mothers in the AHF group had a prolonged clotting time (over 12 min) The same was true of all three mothers in the Christmas group

Two mothers of patients with double defect also had a prolonged clotting time Of two mothers of Hageman patients one had a prolonged and the other a normal clotting time A mother of a patient with PTA deficiency had a normal clotting time

dogs with haemophilic dogs bred female animals half of which were haemophilic By mating these haemophilic she dogs with haemophilic he-dogs the total offspring became haemophilic as might be expected according to Nasse s law

Instances are given in the literature of haemorrhagic diathesis in women whose blood shows impaired thromboplastic activity owing to a lowered AHF concentration usually associated with a reduced capillary resistance (*Alexander & Goldstein* 1953 *Larrieu & Soulier* 1953 *Creveld Jordan Punt & Veder* 1955 *Matter Newcomb Melly & Finch* 1956 *Nilsson Blomback Blomback & Svennerud* 1956 *Nilsson Blomback & von Francken* 1957, *Jurgens Lehmann Wegelius Erikson & Hiepler* 1957) None of these haemorrhagic diatheses have anything to do with true haemophilia Some of them are found in families with von Willebrand Jurgens disease (*Matter Newcomb Melly & Finch* 1956 *Jurgens Lehmann Wegelius Erikson & Hiepler* 1957)

Similar pictures have been described in cases of women whose thromboplastic activity in the blood was reduced owing to deficiency of the Christmas factor (*Sjølin & Videbæk* 1956 *Hardisty* 1957)

True haemophilia in women has not yet been demonstrated in this country

The Haemophilic Transmitter
The Bleeding Tendency

The bleeding tendency in the heterozygous female carrier the transmitter has been described in detail in the literature within the last few years by *Andreassen* (1943) *Schmid* (1951) *Merskey & Macfarlane* (1951) *Neiger* (1951) *Pianta* (1953) and *Sjølin* (1954) among others The figures stated for the incidence of an abnormal bleeding tendency among the transmitters differ considerably ranging from 50 per cent (*Pianta* 1953) to 14 per cent (*Andreassen* 1943) The bleeding tendency in transmitters is only in rare cases so strong as to evidence the presence of a haemophilic gene (*Andreassen* 1943) Hence a bleeding tendency is a bad criterion for deciding whether or not a woman is a transmitter

The Clotting in Transmitter Blood

The interest in tracing the transmitter by investigating the clotting of the blood goes back to *Weil* (1906) who presumably was the first to demonstrate a prolonged clotting time in the mothers of haemophiliacs Up to 1943 the problem was investigated especially by German workers (see *Andreassen* 1943 *Sjølin* 1954) generally with the result that a varying but large proportion of the transmitters of haemophilia were found to have a prolonged clotting time

Andreassen (1943) using Burker s method found a prolonged clotting time in 30 out of 31 transmitters

Chapter XIV

HEREDITY

Introduction

In this country the genetic relations of haemophilia have previously been a subject for thorough investigation (*Andreassen* 1943) Hence only a few problems of genetic interest will be discussed below Be it mentioned in this connection that a renewed study on the fertility of the haemophiliacs and the female transmitters is being contemplated in collaboration with Dr Mogens Hauge the University Institute for Human Genetics

Mode of Inheritance

It is universally agreed that *classical haemophilia* is inherited according to *Nasse*'s law (1820) i e women belonging to haemophilic families transmit the disease from the haemophilic fathers to their offspring even if they marry healthy men The disease is in other words inherited as a sex linked recessive character The women themselves never exhibit any abnormality (see however p 151)

The inheritance of *Christmas disease* is likewise that of sex linked recessivity in all the cases published so far with attending pedigrees (*Biggs Douglas Macfarlane Dacie Pitney Merskey & O Brien* 1952 *Lewis & Ferguson* 1953 *Frick* 1954 *R L Rosenthal* 1954 *Koller* 1955 a)

Verstraete & Vandenbroucke (1955) have reported a case of haemophilia with *double defect* (deficiency of the AHF plus Christmas factor) Two maternal male cousins had AHF deficiency alone *Sjølin* (1957) has described a family (family 1 of the present work) in which there were found two patients with combined haemophilia (AHF plus Christmas factor deficiency) Here too the inheritance was that of sex linked recessivity

PTA deficiency which is included among the haemophilic diseases (*R L Rosenthal* 1954 *Frick* 1954) is possibly genetically unrelated to haemophilia being allegedly a dominant disease manifesting itself in both males and females In one family however sex linked recessive inheritance has been found (*Stefanini & Dameshek* 1955)

Rosenthal Dreskin & Rosenthal (1955) arrived at the result that the dise

Fig 74

Clotting times in 18 women having haemophilic sons

Clotting defect of sons				
AHF	Christmas factor	AHF+Christmas factor	PTA	Hageman
5½–11½	7½–13	6–13	6½–11	8½–13½
6 –12½	6 –13½	7–13		7½–10½
6 –13½	6½–13½			
7 –13½				
7½–13½				
7½–13½				
6 –14				
6 –15½				
–17				
8 –17½				

This small series allows of limited conclusions only but it is evident that many of the transmitters in the families representing the most frequent and severe forms of haemophilia i e classical haemophilia and Christmas disease may have a prolonged clotting time

Thrombin Generation in Transmitters

The thrombin generation was tested in a few mothers of haemophilic patients

In three (family 105 as well as families 80 and 112) belonging to families with AHF deficiency and the Hageman trait respectively the thrombin generation was normal

In one (family 53 no 1) the thrombin generation was poor This plasma was unable to normalise the thrombin generation in plasma from the son who had Christmas disease though some improvement was obtained The ability of this transmitter plasma to form thrombin became perfectly normal after the plasma had been stored at –20° C for 3 months being now able to normalise the thrombin generation in the son s plasma

As it is of great importance from a genetic hygienic aspect to trace the transmitter of haemophilia the whole problem should be submitted to further investigation using newer methods within coagulation research

Thrombin Generation in Non Transmitters

In some of the haemophilic families the author investigated the thromboplastic activity in plasma from women who either had no children or only healthy children

An abnormal thromboplastic activity was found in none of these They all had a normal recalcification time and a normal clotting time

mothers of the haemophiliacs suggesting that the gene for haemophilia had been transmitted from the mother to her son Further two women who were sisters of haemophiliacs had a prolonged clotting time a fact which likewise argues in favour of hereditary haemophilia in these cases

In this connection we must also bear in mind that the gene for haemophilia can be inherited from one transmitter to another through two or more generations without haemophilic sons being born (*Quick* 1957) In the Swiss Tenna family which has Christmas disease the gene for haemophilia has been seen to pass five female generations before it manifested itself again *(Verschuer)* *Pianta* (1953) has described a haemophilic family in which the disease had not manifested itself for 230 years

Present Investigations

Solitary cases of haemophilia have been found in 25 of the Danish haemophilic families

Of the 20 families included in *Andreassen*s series (1943) in which sporadic haemophilia occurred families 10 29 42 57 58 60 and 63 have living haemophilic members Haemophilia continues to occur sporadically in families 29 (where the patient apparently is well though) as well as in 42 57 58 60 and 63 The latter family has not been included under sporadic haemophilia however because patient number 1 is stated to have had prolonged haemorrhage after dental extraction and a tendency to epistaxis In family 10 there are now three haemophiliacs Two of these were alive in 1943 The inheritance is sex linked recessive In the remaining families no other haemophiliacs had been born at the time of conclusion of the present investigation The patient in family 42 has no children The patient in family 57 has a son and a daughter In this latter family there is a chance that the daughter may bear haemophilic sons The patient in family 58 has no children while the patient in family 60 has three sons If this patient gets no daughters no more haemophiliacs will be born in this branch of the family

The clotting defects of the 25 living patients with sporadic haemophilia are distributed as shown in the table fig 75

Fourteen lack the AHF three the Christmas factor four the AHF plus Christmas factor one the Hageman factor two the AHF plus freezing/serum factor while one has no demonstrable clotting defect

Thus all clotting defects except PTA deficiency are represented among the patients with sporadic haemophilia

The figures stated for the incidence of sporadic haemophilia differ somewhat

In a leading article in the British Medical Journal (1956) it is stated that 30–40 per cent of the total number of haemophilic cases are sporadic

Biggs & *Macfarlane* (1957) set the incidence of sporadic haemophilia at

ase is autosomally dominant and that accordingly there is a 50 per cent chance that a carrier of the disease will transmit this

No more than a single investigation is available into the inheritance of the symptom free *Hageman trait*

Margolius jr & Ratnoff (1956) examined 44 near relations of two sisters with the Hageman trait without being able to demonstrate the defect in any of these 44 more particularly no signs of the Hageman trait were found in three children and four grandchildren of the two affected sisters The gene thus seems to be recessive

Sjølin (1957 d) has reported a family (family 80 of the present work) in which a patient with signs of haemophilia lacked the Hageman factor The maternal grandfather of this patient had had a mild degree of haemophilia but had not had his clotting defect classified The mother of the propositus displayed no signs of a clotting defect The inheritance thus seems to be that of sex linked recessivity

Present Investigations

In 45 out of 80 investigated Danish families with haemophilia the disease was inherited according to Nasse s law, i e as a sex linked, recessive disease

This was independent of the type of clotting defect whether AHF deficiency (e g families 2 and 13) Christmas factor deficiency (e g families 4 and 22) AHF plus Christmas factor deficiency (e g family 1) the Hageman trait (e g families 80 and 112) or deficiency of the AHF plus serum/freezing factor (e g family 39)

The PTA defect was observed in two generations in family 109 Sex linked recessive inheritance was seen here too None of the female members of the two families under review with PTA deficiency have had any signs of a haemorrhagic diathesis The mother of one of the patients (family 106) had a normal clotting time

The problem of different defects within the same family will be discussed later

According to the results of the present investigation all haemophilic diseases i e the pure plasmathromboplastic defects seem to be due to a sex linked recessive gene

Sporadic Haemophilia

By sporadic haemophilia we mean isolated cases of haemophilia in families with no other instances of the disease

So far sporadic haemophilia has not been found to differ from hereditary haemophilia with regard to symptomatology clinical course or haematology (*Andreassen* 1943 *Neiger* 1951) In four families supposed to have instances of sporadic haemophilia *Andreassen* found a prolonged clotting time in the

Fig 76

The clotting defects in 10 families having haemophilia in a single sibship only

Family	Number of haemophilic brothers	Alive	Lacking
25	4	3	AHF + serum/freezing factor
53	3	1	Christmas factor
54	3	2	AHF
59	2	-	AHF + serum/freezing factor
6-	2	1	Christmas factor
70	-	2	1) AHF 2) Thromboplastin inhibitor
78	3	3	AHF
79	2	2	AHF
105	2	-	AHF
106	2	1	PTA
	-5	19	

tients too The clotting defect was found to be the same within the individual sibship except in family 70 where one patient had AHF deficiency and another thromboplastin inhibitor The latter may however have developed in consequence of transfusions in a patient with AHF deficiency

Mutation is an unlikely explanation of this phenomenon More likely the gene for haemophilia has arisen in the mothers or the female ancestry of these in direct line

Haemophilic Families with Multiple Clotting Defects

Only four reports are available in the literature on simultaneous occurrence of different clotting defects within the same haemophilic family

Koller (1955 a) mentions demonstration of AHF deficiency and Christmas factor deficiency in two different patients of the Zuricher Oberland family *Scardigli & Guidi* (1956) have described a family in which one patient had PTA deficiency while his mother s brother had AHF deficiency Finally *Verstraete & Vandenbroucke* (1955) and *Sjölin* (1957 c) have reported on families in which there were found patients with AHF plus Christmas factor deficiency as well as patients with AHF deficiency alone

No satisfactory genetic explanation has been given as yet regarding these facts

Brinkhous & Graham (1954) divided the AHF defects into four groups according to the severity of the clotting defect to each of which they attributed an allelomorph on the X-chromosome The gene for Christmas disease is in

Fig 75

Distribution of the clotting defects in 25 families with sporadic haemophilia

Defect	Family no	Number
AHF	57 60 69 67 72 73 82 87 91 94 100 103 104 110	14
Christmas	85 86 102	3
AHF + Christmas	42 58 77 95	4
Hageman	90	1
AHF + freez /se	75 108	2
No defect	29	1
		25

25–30 per cent *Andreassen* found 20 sporadic cases among 205 i e a scant 10 per cent Calculated on the basis of the patients then alive the incidence is 14 out of 81, or about 19 per cent

In the series under review there were found 25 solitary cases of haemophilia among 148 investigated i e about 17 per cent

The low incidence of sporadic haemophilia in the Danish series is presumably due to the better chances of collecting sufficient pedigrees and tracing and examining relatives in a small country than in a large one

As the solitary cases of haemophilia differ neither clinically haematologically nor genetically from other haemophilic states and as the same clotting defects are represented among these patients as among other haemophiliacs it is unnecessary to preserve the designation of sporadic haemophilia which only serves to confuse non experts Since haemophilia as stated previously (see p 115) may run an almost symptom free course with no demonstrable clotting defect for long periods the healthy members of the family concerned will have to be examined more closely before it can be said with reasonable certainty whether the case in question is one of sporadic haemophilia Sporadic haemophilia is probably in many instances referable to an inadequate family history The remainder may be due to mutation either in the haemophiliacs themselves or in their female ancestry

Families with Haemophilia in a Single Sibship

In continuation of the description of sporadic haemophilia mention will be made of families in which there are or have been not less than two haemophilic brothers in a single sibship but no other cases of the disease neither among the mother s siblings nor among the offspring of these the siblings of the maternal grandmother etc

These are the following ten families (see the table fig 76)

It is seen that different clotting defects are represented among these pa

tient no 4 would have received only one of the defective genes The pathogenic genes can no more have been located at different sites on the X-chromosome because if so the father of transmitter no 1 would have been affected

The only possibility left is that the pathogenic genes are carried on the same X-chromosome (see fig 78) in transmitter no 1 Half of her sons would

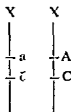


Fig 78

then inherit a double defect while the other half would be healthy Half of her daughters would be transmitters of a + c̄ and the others healthy but none could be transmittors of a or c̄ alone unless crossing-over had taken place Transmitter no 3 could only get a son with AHF deficiency alone if crossing over had occurred at the formation of her own sex cells or of some of her mother's sex cells

Family 8 In this family three brothers nos 3 4 and 5 have haemophilia Number 3 lacks the Hageman factor while the two others have slight Christmas factor deficienccy

Supposing that the Hageman trait is due to a third pathogenic gene d̄ this must be carried on the same X-chromosome as c̄ as it cannot be allelomorphic to c̄ If it were allelomorphic to c̄ the mother would presumably have been a haemophiliac which she is not Her father being healthy the pathogenic genes cannot have been located on separate chromosomes either (see fig 79)

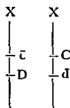


Fig 79

This possibility cannot be entirely excluded however in case he has an asymptomatic Hageman trait As it has been mentioned previously that the Hageman trait in cases causing signs and symptoms also seems to be inherited as a sex linked recessive character it plays no part whether the father of the

their opinion not allelomorphic to these four, but carried elsewhere on the X chromosome

According to *Koller* (1955 a) the genes protecting against AHF deficiency and Christmas factor deficiency respectively are located at different sites of the X chromosome Occurrence of combined defect (AHF plus Christmas factor deficiency) would then be extremely rare the incidence of AHF deficiency being 1 in 100 000 and that of Christmas factor deficiency about 1 in 1 000 000 (according to *Verstraete* 1955) The fact that among about 300 haemophilic patients classified so far six suffered from AHF plus Christmas factor deficiency therefore militates against Koller s hypothesis

The Danish Material

Different clotting defects in the same family (see the table fig 77) have been demonstrated in seven families of the present series comprising 27 investigated patients

Family 1 In this family the three investigated patients belong to three different generations Patient no 8 from the fifth generation behaves clinically and haematologically (lacks only the AHF) differently from the patients nos 4 and 9 who have AHF plus Christmas factor deficiency

If we attribute deficiency of the Christmas factor and deficiency of the AHF to two pathogenic genes ($\bar{c}$ and a respectively) transmitter no 1 must possess both genes Even if her husband had had haemophilia he could not have transmitted a pathogenic gene carried on the X chromosome to his sons The two pathogenic genes cannot be allelomorphic in transmitter no 1 because if so she would herself have haemophilia and would then not have carried normal allelomorphs (C and A) to suppress the pathogenic genes while pa-

Fig 77

Seven haemophilic families with different clotting defects within the individual family

Family no	Defect: AHF	Christm	AHF Chr tm	AHF+ [illegible]	PTA	H g	Number of patients
1	8		4 9				3
8		4 5 6 8				3	5
36		2 3				4	3
39	24 25			21 23 26			5
51		3				4 5	3
88	2 3 4					5	4
101	2 4 5			3			4
						Total	27

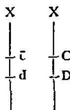


Fig 82

The above reflections also apply where *family 51* is concerned as trans mitter 1 and her father do not have manifest haemophilia

In *family 88* patient no 2 may lack the AHF and the Hageman factor The crossing over must then have taken place at the sex-cell formation of the daughters It is impossible to say for certain whether this double defect is present If the patient lacks only the AHF and his wife has an asymptomatic Hageman trait the two heterozygous daughters may have the combination shown in figure 83 This may explain the occurrence of both AHF deficiency and

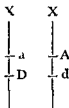


Fig 83

deficiency of the Hageman factor in the grandchildren of patient no 2 The fact that one has healthy sons must be due to crossing-over The two daughters cannot have two pathogenic genes a and d̄ at the same site as they would then be haemophilic which they are not

Family 101 Patient no 3 has AHF deficiency plus slight Christmas factor deficiency while the remaining haemophilic family members lack the AHF alone The mother of nos 2 and 3 is healthy and may therefore have a gene structure as shown in fig 84 She can in conformity with the current rules of

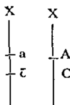


Fig 84

three patients has an asymptomatic Hageman trait because his potential pathogenic gene carrying X chromosome is not transmitted to the sons

If we leave out of account the possibility that the maternal grandfather of the three patients has an asymptomatic Hageman trait, we can if we maintain the hypothesis of two non allelomorphs, only explain the different defects in the three brothers by crossing over in the mother s sex cell formation The possibility might also be conceived however that the Christmas factor deficiency in nos 4 and 5 concealed a Hageman trait which would be very difficult to disclose

Family 36 Two brothers, nos 2 and 3, lack the Christmas factor, and the third no 4 the Hageman factor The latter patient, who is less troubled by his disease than his brothers has a different father and no information is available as to whether he has the Hageman trait This probably makes no difference however because the gene for the Hageman trait $\bar{d}$, is carried on the X chromosome, which the patient has inherited from his mother

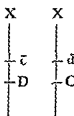


Fig 80

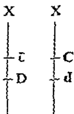


Fig 81

The mother can be said for certain not to have the gene combination shown in figure 80 as she would probably then have haemophilia which she has not If her father who was healthy had an asymptomatic Hageman defect she may have the gene combination shown in figure 81 and this may account for the occurrence of two different defects in the sons If she has the gene combination in figure 82 the different clotting defects in the sons can only be explained by crossing over

In families where only one clotting defect has been demonstrated considerable variations may be seen in the manifestation of the disease In family 2 for instance patient no 11 was considerably more ill than the remaining affected members of the family The same is true of patient no 19 in family 22 In family 10 patient no 3 is troubled but little by his disease compared with the two other haemophiliacs of the family

The expressivity of the gene for haemophilia may vary not only from one patient to another in the same family but also in the individual patient as mentioned in the section on the variability of the clotting defect It is also well known from clinical practice that the state of health of the individual patient varies considerably

Different clotting defects within the same family were as stated demonstrated in seven families comprising 27 living haemophiliacs i e on an average barely four investigated patients per family In the remaining 71 families where only a single clotting defect was diagnosed the number of patients investigated averaged barely two

If we deduct the 25 families with solitary cases of haemophilia the average number of investigated haemophiliacs in each of the remaining families was two

In other words two or more clotting defects were observed particularly in the families with the greatest numbers of examined haemophilic patients This goes to show that the phenomenon may be encountered more frequently than previously supposed if the investigations are extended to a greater number of family members

Consequences of the Monogenic Hypothesis

The hypothesis that all haemophilic diseases are referable to a single pathogenic gene the manifestation of which may be influenced by other genes has various consequences

1 *Heredity*

According to *Koller* as well as to *Brinkhous & Graham* patients with a single defect belonging to families in which there are also found combined defects (e g family 39) can only have descendants (in the second generation) with a single defect According to the monogenic hypothesis these patients may very well have descendants (in the second generation) with double defects or other defects This question cannot be definitely settled until the families have been followed through more generations

2 *Mutation*

The incidence of mutation in haemophilia has been calculated by *Andreassen* and by *Dahlberg* (1949) The latter writer estimated that about one third of haemophilic cases arise by mutation

inheritance, give birth to a son with double defect, but if she gets a son with AHF deficiency alone, a crossing over must have taken place at the formation of some of her sex cells Her daughter has two haemophilic sons with a single defect (AHF) This daughter may then either carry a pathogenic gene on her X chromosome or, in case she has the same gene structure as her mother a crossing over must have occurred at her sex cell formation

The mother of nos 2 and 3 cannot carry two pathogenic allelomorphs as she would then have been ill

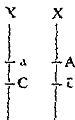


Fig 85

She may have the gene combination shown in *figure 85* if *her mother* was a transmitter of a or c̄ In that case she can only have a son with double defect if crossing over has taken place

In the case of *family 39* the arguments adduced above can be used Supposing that each clotting defect is due to a separate pathogenic gene sons with a single defect can only be born to the transmitters nos 17 and 18 if crossing over has occurred Pathogenic allelomorphs in the mothers are out of the question the mothers being healthy

The above arguments seem to justify the conclusion that the different forms of haemophilia are not due to different pathogenic genes If they were the following two possibilities would exist The genes might be present as allelomorphs in the mothers of the haemophiliacs with double defect In that case these mothers would have haemophilia but such has not been observed in the present investigation The other possibility is that the patients might carry two or more non allelomorphs This is also rather unlikely because of the observed rather high incidence of double defects which would then have occurred exclusively by crossing over

The number of combined defects (24) in the present series is much greater than might be expected if each defect were due to a separate pathogenic gene

The hypothesis may therefore be advanced that the various haemophilic diseases are due to a single pathogenic gene which manifest itself in different ways owing to the presence of different modifying genes in the individual patients

This view is supported by certain other facts

CONCLUDING REMARKS

Haemophilia by which we understand a hereditary haemorrhagic diathesis caused by defects of the plasma thromboplastin presents a very polymorphous clinical picture The manifestation of the disease varies more than commonly supposed from the severest degrees with very frequent profuse haemorrhages to cases with only very slight signs and symptoms allowing the patients to fill a post satisfactorily

Correspondingly the blood analyses have revealed great variations in the coagulability of the blood not only from one family to another but also from patient to patient within the same family Variations in the blood coagulability have even been demonstrated in the individual patient The causes of these variations are unknown One of the most important future tasks must be that of investigating the factors influencing the plasmathromboplastic activity

The influence of freezing on the coagulability in some of the clotting defects is of more than theoretical interest If the effect of freezing can be produced otherwise this will open out therapeutic possibilities beyond the present fairly unsatisfactory substitution therapy

No satisfactory explanation can be given of the fact that the platelets of haemophilic blood after having been washed in physiological saline can in some instances normalise the inhibited clotting

Comparative studies on the coagulation factors existing in plasma and serum and their interactions are also urgent

In the present work the pathogenesis of haemophilia has been submitted to a one sided investigation from the view that the haemorrhagic diathesis is due to deficiency of coagulation factors However the question of the role of the naturally occurring inhibitors also suggests itself Although papers dealing with these problems are available the questions of inhibitor plasmathromboplastin defect have not yet been co-ordinated in a satisfactory way

Another question suggesting itself is whether haemophilia is due exclusively to defects of the thromboplastic components of plasma

Johnson Seegers Koppel & Olwin (1957) found that prolonged treatment with anticoagulants may cause a fall of the Christmas factor concentration to zero without this giving rise to such signs of a bleeding tendency as in haemophilia

Most of the previously described Hageman patients who have a grave clotting defect display no signs of a haemorrhagic diathesis Those described

The incidence of mutation has not been calculated in the present work, because the haemophilic diseases have not yet been proved to be due to one single pathogenic gene

3 *Eugenics*

One of the most important problems here is that of tracing the transmitter As stated previously this problem has not yet been solved to satisfaction Especially it must be regarded as insufficient to establish the presence of heterozygosity on the basis of a single clotting test, because the clotting defect may probably vary appreciably in the transmitters just as it has been shown to do in the haemophiliacs

4 *Prognosis*

It is mentioned in the introduction that a study of the prognosis of the various haemophilic diseases has been aimed at As stated previously under the individual defects this has been possible *quoad functionem* but not *quoad vitam* because we have no knowledge of the quality of the clotting defect in the earlier haemophilic patients

SUMMARY

Recent investigations into the clotting defect in haemophilia have shown that this disease is divisible in various subgroups

Denmark being a relatively closed territory with regard to population it was found to be a matter of considerable interest to have all Danish haemo philiacs examined with a view to the clotting defect and to obtain a general idea of the symptomatology and prognosis of the individual forms of haemophilia Such a study would also afford a basis for procuring information on the rules according to which the various clotting defects are inherited a question which the literature available does not clarify

Our present knowledge concerning the clotting of blood has develophed through five periods *(Chapter I)*

The *first period* ended in 1905 with the advancement of Morawitz theory of coagulation according to which the blood clotting takes place in two phases

First phase Prothrombin + thrombokinase + Ca ——→ thrombin
Second phase Thrombin + fibrinogen ——→ fibrin

The *second period* comprises the next 30 years during which the tissue thromboplastin was studied This consists of a lipoid component bound on protein

Within this period other theories were advanced concerning the blood clot ting e g by *Howell Mills & Quest Nolf* as well as *Stuber & Lang*

Within the *third period* some American writers reported on investigations the results of which bore out Morawitz theory *Patek & Taylor* found the anti haemophilic globulin while *Dam & Schønheyder* detected the vitamin K and demonstrated its importance in the prothrombin synthesis

The *fourth period* was concerned with mapping of the factors of the pro thrombin complex

Quick (1943) demonstrated a labile factor and independently *Owren* (1944) described a haemorrhagic diathesis caused by deficiency of proaccelerin which is identical with the labile factor Proaccelerin is indispensable for the conversion of prothrombin into thrombin the rate of this process and the amount produced of thrombin depending on the presence of proaccelerin

A few years later *Owren* and *Koller* detected a factor proconvertin which likewise plays an important part in the activation of the tissue thromboplastin

in the present work all have a very mild clotting defect, but nevertheless they display signs of a haemorrhagic diathesis These two groups possibly differ completely a problem which can be clarified only by comparative investigations of the two groups of patients In the group under review other pathogenic factors are probably involved besides the clotting defect of the plasma

Sjølin & Astrup (1958) recently demonstrated a powerful thromplastin inhibitor in the synovial capsule of a haemophilic patient (AHF deficiency) at a time when the clotting defect of his plasma had been corrected by transfusion If the function of the tissue thromboplastin in haemophiliacs is obstructed by an inhibitor this can explain the diffuse tissue haemorrhage in the haemophilic patients The influences of the various tissues on the local haemostasis should therefore be submitted to extensive investigation preferably on haemophilic animals

clotting defects As will be mentioned later this defect can be compensated after addition of normal or haemophilic blood platelets

Investigations of the plasma thromboplastin in cases of haemophilia have revealed deficiency of the antihaemophilic factor the Christmas factor or the plasma thromboplastin antecedent or concurrent deficiency of the two first mentioned factors

Finally circulating acquired anticoagulants have been detected in the blood of some haemophiliacs

The methods of detecting blood clotting defects may be conveniently divided into three groups *(Chapter III)* as shown indirectly by Morawitz scheme

The first group of methods is employed for investigating the last phase of the disturbed coagulation process

This group is mainly concerned with fibrinogen determination which can be performed by numerous methods and detection of inhibitors The latter are most simply demonstrable by their ability to prolong the clotting time of normal blood

The second group serves to demonstrate defects of the prothrombin complex (proaccelerin proconvertin and prothrombin) The easiest method is that of *Quick* s prothrombin time determination Of other methods mention may be made of *Owren s* prothrombin proconvertin time determination where the coagulation system is stabilised with regard to proaccelerin fibrinogen and inhibitors

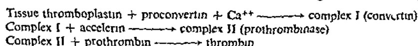
The third group is employed for investigating the plasma thromboplastin after having made sure that the components of the prothrombin complex and the fibrinogen content of the tested blood are normal

For the *thromboplastin generation test* a mixture of fresh, adsorbed oxalated plasma (containing AHF PTA Hageman factor and proaccelerin) is incubated with serum (containing Christmas factor PTA Hageman factor and proconvertin) and a platelet suspension After recalcification of the mixture a specimen of this is transferred at intervals of one minute to a sample of platelet free citrated plasma recalcified simultaneously and the clotting time is determined Defects of the incubation mixture will give prolonged clotting times Substitution experiments using normal substrates will generally make it possible to detect the clotting defect and the thromboplastin generation test has in fact been extensively used for classification of the haemophilic diseases The objection may be raised to the method that it works with such unphysiological substrates as serum and washed platelets

The *prothrombin consumption test* is used to measure the prothrombin activity in serum Normally 85 per cent of the prothrombin has been converted into thrombin by the end of the clotting In haemophilic blood on the other hand the conversion of prothrombin occurs at a very slow rate The pro

whereas it is doubtful whether it has any influence on the function of the plasma thromboplastin (*Ackroyd Seegers & Johnson*)

In *Owren*s opinion thrombin is formed according to the following schem



Tissue thromboplastin + proconvertin + Ca^{++} ——→ complex I (convertin)
Complex I + accelerin ——→ complex II (prothrombinase)
Complex II + prothrombin ——→ thrombin

Within recent years one more factor has been demonstrated the Stuart Prower factor which is also essential for the conversion of prothrombin into thrombin

During the *fifth period* the thromboplastin of the plasma itself was mapped The plasma thromboplastin is a function of several factors A clarification of these problems has been obtained particularly through investigations into the clotting defect in haemophilia

Collingwood & MacMahon stated as their opinion that the platelets contain a precursor of thromboplastin prothrombokinase which is liberated at the beginning of the clotting

Quick and *Brinkhous* showed that the platelets can only accelerate the clotting in the presence of the antihaemophilic factor

In 1952 a factor the Christmas factor was found almost simultaneously in the U S A and in Great Britain This is necessary for the normal clotting

Another factor which likewise is indispensable for the normal blood clotting is the plasma thromboplastin antecedent (PTA) detected in 1953

The existence of factor X was first demonstrated in 1954 and that of the Hageman factor in 1955 These are both believed to be included in plasma thromboplastin

Our present knowledge calls for the modification shown in fig 3 p 19 of Morawitz scheme which originally did not comprise plasma thromboplastin

Haemophilia is characterised haematologically by 1) a prolonged (or normal) clotting time 2) a normal prothrombin time and 3) delayed conversion of prothrombin into thrombin *(Chapter II)*

The contents of fibrinogen prothrombin and calcium in haemophilic blood have never been found definitely abnormal

It is also universally agreed that the thromboplastic activity in the tissues of haemophiliacs is normal However the results of *Lowenburg & Rubenstone*s investigations suggest that an inhibitor exists in the thyroid body and the liver a fact to which little attention seems to have been given

The number of thrombocytes is stated to be normal in haemophilic blood but there is some disagreement as to whether their function is normal Some writers claim to have demonstrated increased stability of the blood platelets of the haemophilic patients These discrepancies may be due to the various investigators having worked with haemophilic blood samples having different

Storage of citrated plasma at 4° C was found to raise its thromboplastic activity

Freezing likewise accelerates the thromboplastic activity presumably owing to damage of the platelets

A rise of the number of thrombocytes accelerates the thrombin generation and raises the thrombin concentration

Addition of heated readsorbed serum or normal serum to normal plasma raises the rate of thrombin generation as well as the maximum thrombin concentration obtained

Adsorbed bovine plasma adds slightly to the amount of thrombin formed but does not accelerate the thrombin generation

A considerably increased thromboplastic activity is obtained by removing the platelets of the plasma washing them in saline and adding them again

In plasma from patients under dicoumarol treatment where the prothrombin proconvertin level is lowered the amount of thrombin generated is reduced

Chapter VI is concerned with the collection of the material There were found 156 patients with haemophilia or 1 per 27 000 inhabitants in Denmark or 1 per 14 000 male inhabitants

As the blood samples from many of the patients had to be stored in ice water for up to 20 hours after the sampling the influence of such short storage on the thromboplastic activity of haemophilic plasma was investigated In plasma lacking the AHF or the Christmas factor or both storage caused no unquestionable change of the thromboplastic activity In plasma lacking the Hageman factor on the other hand the thrombin generation became normal and in cases of mild degrees of AHF deficiency the thromboplastic activity improved somewhat

Chapter VII deals with the diagnosis of haemophilia and the problems of differential diagnosis Haemophilia is characterised by a bleeding tendency a defect of the plasma thromboplastin and by the sex linked recessive inheritance

The differential diagnosis is from other hereditary haemorrhagic diatheses which may be 1) plasmatically induced 2) thrombocytogenic or 3) vascularly induced In group 2) a diminished content of AHF in the plasma has been demonstrated within recent years

Regarding the symptomatology it is emphasized that it is impossible to distinguish clinically between classical haemophilia (AHF deficiency) and Christmas disease It is universally agreed that manifest bleeding is rare in cases of PTA deficiency Among the cases of the Hageman trait published so far only one is reported to have given rise to pronounced signs and symptoms

thrombin consumption test has been extensively used to classify the haemophilic diseases

In mild cases of haemophilia the method cannot disclose the clotting defect

The most frequently employed method so far for investigating haemophilic blood is that of *clotting time determination* The method is not specific as all pronounced defects of the coagulation system cause a prolonged clotting time Investigations of fairly large series of haemophiliacs have shown that up to 20 per cent had a normal clotting time According to the literature there is no constant correlation between the length of the clotting time and the severity of the clinical signs

Finally the *thrombin generation test* is described The procedure is that of transferring at intervals small samples of clotting plasma to a fibrinogen solution The clotting time in the solution is determined this being an indirect expression of the thromboplastic activity of the plasma provided one has made sure in advance that the components of the prothrombin complex are normal and that no inhibitors are present This method has hitherto been employed to a moderate extent only for classification of the haemophilic diseases

The following methods were chosen for investigating the Danish haemophilic patients Recalcification time determination and the thrombin generation test in citrated plasma *(Chapter IV)* These investigations were supplemented by thrombocyte counts and determination of Quick s prothrombin time

The following test substrates were used for classification of the clotting defect Adsorbed bovine plasma normal human serum heated adsorbed human serum and platelet suspensions Further the influence of freezing on the coagulation process was investigated in several cases Finally the mutual influences of different types of haemophilic plasma on the coagulation process were studied

The normal thrombin generation test is described in *Chapter V* The thrombin generation strats between 0 and 4 minutes (lag period) after recalcification of the plasma The thrombin concentration reaches maximum from 2 min 30 sec to 8 min 45 sec after the recalcification The inactivation of the thrombin is complete 6 to 18 minutes after the recalcification

In the control material the recalcification times lie under 4 min 40 sec

Repeated thrombin generation tests on plasma from the same normal subject revealed some variation of the maximum thrombin concentration obtained and the time at which it was reached This is presumably in the first place due to variations in the thromboplastic activity of the plasma and less to variations in the fibrinogen used

Repeated tests on the same plasma sample gave only small variations of the thrombin generation and the recalcification time

defects these 11 could not correct the clotting defect in plasma with AHF deficiency

The average age of this group of patients was 33 In nine the bleeding tendency had manifested itself before the age of 3 years

All the patients had had bleedings into the skin (see fig 34) Seven had had haemarthroses Out of five adults with haemarthroses three had perma nent joint deformities Five declared that the disease had grown milder in the course of years The nine adults were all fully or partially fit

The recalcification times ranged between 4 min and 7 min 25 sec The thrombin generation test showed normal conditions in two In the remainder considerable amounts of thrombin were formed after a prolonged lag period (see fig 35)

In six out of seven cases the thrombin generation became normal after addition of frozen platelet suspension

In four cases fresh platelet suspension was added and this gave a normal thrombin generation in all four samples

In seven out of eleven cases the thrombin generation became normal after the plasma had been frozen

In five cases the thrombin generation was investigated in platelet poor plasma (1000–7000 thrombocytes per cubic millimetre) which had been frozen down In three of these the thrombin generation became normal

No explanation can be given as yet of how serum can normalise the clotting in a plasma with slight AHF deficiency Maybe serum contains small amounts of AHF Another possibility is that factors having a weak AHF effect are formed (from the thrombocytes?) in plasma during its conversion into serum The latter hypothesis may perhaps be supported by the results of the ex periments with freezing and addition of platelets

A diagnosis of *Christmas disease* was made in 27 patients (18 per cent) The defective thromboplastic activity became normal after addition of serum but not after addition of adsorbed bovine plasma

The average age for these patients was about 27 years

The first abnormal bleeding occurred in the period between birth and 16 years of age on an average at the age of 2 years

All the patients had had bleedings into the skin 21 patients had had haemarthroses which in 15 out of 17 adult patients had left permanent changes (see also the table fig 36) Seven patients stated that the disease had grown milder with increasing years About one third of the adult patients were disabled Eight adults could support themselves Two patients had done their military service

The recalcification times ranged between 4 min and 20 min

In 12 patients hardly any thrombin was formed in the plasma In 14 some thrombin was formed but only after a prolonged lag period In one patient the thrombin generation and the recalcification time were normal

The results are given of a classification of the clotting defect in 148 out of 156 haemophilic patients, and the criteria are stated on the basis of which the individual defects have been defined

Deficiency of the AHF plus Christmas factor was demonstrated in 6 patients

AHF deficiency and slight Christmas factor deficiency 18 patients

AHF deficiency alone 61 patients

Deficiency of the Christmas factor alone 27 patients

Slight AHF deficiency 11 patients

Slight deficiency of the Christmas factor 6 patients

Deficiency of the Hageman factor 11 patients

PTA deficiency 3 *patients*

In one patient there was found a circulating anticoagulant In three (from *Andreassen*s series) no clotting defect whatever was demonstrable

Finally blood from one patient showed slight deficiency of the AHF plus Christmas factor

The average age for the 61 patients (40 per cent) with *isolated AHF deficiency* (classical haemophilia) was 19 years The first abnormal bleeding occurred before the age of 10 in 44 already within the first year of life

An account is given of the symptomatology (see fig 33) Ninety two per cent of the patients had had haemarthroses of whom 59 per cent developed permanent joint deformities 25 per cent declared that the bleeding tendency had grown milder with increasing years One third of the adults were able to support themselves One third were partially fit while one third were unfit for manual work

The clotting defect was corrected by adsorbed bovine plasma but not by serum In plasma from eight of the patients the clotting defect could also be corrected by adsorbed serum This group does not differ clinically from the remaining patients with AHF deficiency

The recalcification times ranged between 4 min 45 sec and 16 min

The thrombin generation was very poor in this group except in seven where appreciable amounts of thrombin were generated but after a prolonged lag period

The thrombin generation became normal in one out of eight plasma samples in which this was tested after the plasma had been stored at –20 C The effect of added platelet suspension was investigated in nine plasma samples In two of these the thrombin generation became normal

In some instances there was disagreement between the clotting defect and the clinical picture suggesting an action of other pathogenic factors in haemophilia besides the clotting defect

The group of *slight AHF deficiency* comprised 11 patients Eight of these had primarily been classified under PTA defects and three under Hageman defects However in cross tests with plasma samples having known clotting

PTA or Hageman defects Control tests with plasma samples having known AHF or Christmas factor deficiency were found to be necessary

PTA deficiency (Rosenthal s syndrome) was found in three patients The clotting defect in plasma from these patients was corrected by adding either adsorbed bovine plasma or normal serum but not by adding heated read sorbed serum Further the PTA deficient plasma samples were able to correct the clotting defect in both plasma lacking the AHF and plasma lacking the Christmas factor

The two patients had bled only from cuts and after dental extraction One of these had also had haematuria The third patient had had haemarthroses as well which had left permanent joint deformities The two adult patients were fully fit

The recalcification times ranged between 5 min and 9 min 35 sec

In the two mildest cases the thrombin generation was nearly normal In the third the lag period was markedly prolonged

After the plasma had been frozen the thrombin generation was normal in all three cases

In one case the effect of the patient s own serum was tested and found to normalise the thrombin generation

There were no signs of a haemorrhagic diathesis in any of the women belonging to the two families investigated

11 patients were found to lack the *Hageman factor* The clotting defect in plasma from these patients was corrected by adsorbed bovine plasma as well as heated readsorbed serum Plasma samples from these patients corrected the coagulation defect in both AHF deficient plasma and Christmas deficient plasma

The patients ages ranged between 1 and 64 years averaging 29 None had displayed abnormal bleeding phenomena at birth Five were over 7 years of age before they presented signs of a haemorrhagic diathesis

All the patients had had gingival haemorrhages ten had had haemarthroses of whom six were without permanent changes

All the adults were fully fit

The recalcification times ranged between 3 min 50 sec and 8 min

The thrombin generation test revealed considerable thrombin generation but after a lag period of 5–6 minutes

As in the above mentioned substitution experiments the clotting defect could be corrected in the following ways By adding washed thrombocytes to the plasma from which they were derived by freezing the plasma by adding the patient s own serum and in a few instances by adding a fresh or frozen platelet suspension

No inhibitor has been demonstrated in the wash water used for preparing a platelet suspension from one of these patients

In plasma from five out of 21 patients the thrombin generation became normal after it had been stored at –20° C

In 14 vigorously centrifuged plasma samples the thrombin generation did not become normal after freezing of the plasma

Platelet suspension was added in 14 cases In two the thrombin generation became normal

In a few instances the patient s own serum was found to correct the clotting defect of his own plasma This was the case with plasma samples having some thromboplastic activity The clotting defect of such patients cannot be demon strated by the thromboplastin generation test

A fair correlation was found between the severity of the clotting defect and the clinical picture

Clinically it was impossible to distinguish the severe cases of Christmas disease from classical haemophilia and the mild cases of Christmas disease from the Hageman trait

Slight Christmas factor deficiency was diagnosed in six cases These were characterised haematologically by disappearance of the clotting defect after addition of either adsorbed bovine plasma or serum In four the thrombin generation was normalised by heated readsorbed serum These four had pre sumably been classified as Hageman defects and the remaining two as PTA defects However plasma from these patients could not normalise the thrombin generation in plasma lacking the Christmas factor but only in plasma lacking the AHF

The patients ages ranged between 6 and 50 years averaging 23 The bleeding tendency was rather slight Bleedings into the skin and from the alveolar process were the most frequently occurring phenomena No more than three had had haemarthroses of whom one an adult had developed perma nent joint deformities All the adults were fit

The recalcification times ranged between 4 min 45 sec and 6 min 50 sec

The delayed thrombin generation became normal in all six cases after the plasma had been stored at –20 C In two cases vigorously centrifuged plasma was tested in the same way The thrombin generation became normal in both In two cases investigated the patient s own serum was able to compen sate the clotting defect in the plasma from which the serum originated

In one case the thrombin generation became normal after the platelets of the tested plasma had been washed in saline

The six patients of this group were considerably less affected by their disease than those of the preceding group classified under Christmas factor deficiency They also seemed to be less affected than the patients with slight AHF deficiency

It is important to realise that the slight defects cannot be diagnosed solely by means of *normal test substrates* as they will then be characterised as either

No signs of an inhibitor were demonstrated in this group

As the plasma of these patients was unable to correct the clotting defect in Christmas plasma the defect was supposed to be one of AHF deficiency plus slight deficiency of the Christmas factor

These patients were clinically indistinguishable from patients with classical haemophilia

The group of *Miscellaneous* comprised the patients whose haematological pictures rendered them unclassifiable in any of the above mentioned groups

One patient was supposed to have a slight AHF plus Christmas factor deficiency

In one patient a thromboplastin inhibitor was demonstrated

Further in three patients previously diagnosed as haemophiliacs no clotting defect was demonstrable They had all been free from any abnormal bleeding phenomena for several years

Finally a patient is included here who previously was characterised as haemophilic but who was free from any signs and symptoms for several years Only after repeated investigations distributed over a few years was an impaired thromboplastic activity demonstrated in the plasma caused by deficiency of the Hageman factor

In the table fig 51 the Danish haemophilic material has been compared with the comprehensive foreign materials Considerable differences are noted The Danish material presents a relatively smaller number with AHF deficiency a greater number of combined defects more patients lacking the Christmas factor and fewer with PTA deficiency Finally we find in the Danish material 7 per cent haemophiliacs lacking the Hageman factor as well as a small group with no demonstrable clotting defect

Possible causes of these discrepancies are discussed As the most reasonable explanations are stated the better chances of tracing mild cases of haemophilia in a small country and the fact that the thrombin generation test is the most physiological method of demonstrating defects of the plasma thromboplastin using the patient s plasma alone as substrate

The frequencies of the various manifestations of the different clotting defects as well as of the relation between the clotting defect and the working capacity of the patients aged 18 or more have been studied *(Chapter IX)*

The table p 122 gives a survey of these problems

As might be expected the patients with combined clotting defects presented the severest signs of a haemorrhagic diathesis Then followed the groups lacking the AHF and the Christmas factor These are clinically indistinguishable Next followed in decreasing order of frequency slight AHF deficiency slight deficiency of the Christmas factor and finally PTA deficiency and deficiency of the Hageman factor

The chance of the disease growing milder in the course of years proved

The results of the experiments with washed platelets and those with freezing of the plasma suggest that the task of the Hageman factor is to render the platelets labile at the beginning of coagulation and thus accelerate the thrombin generation

No clotting defect was demonstrable in two mothers of Hageman patients

To the group of *combined clotting defects* were referred six patients with deficiency of the AHF plus Christmas factor as well as 18 patients lacking the AHF plus freezing/serum factor Deficiency of the latter factor presumably represents a slight deficiency of the Christmas factor

The ages of the former six patients ranged between 6 and 46 years, averaging 29

The first sign of a bleeding tendency had in five cases occurred within the first year of life All the patients had had bleedings into the skin The five adults had all had haemarthroses which had resulted in permanent changes (see also the table fig 44 p 104)

In none of these patients had the signs and symptoms abated in the course of years

All the adults were disabled

The recalcification times ranged between 10 min 30 sec and 16 min 30 sec The thrombin generation test showed that hardly any thrombin was formed The thrombin generation became normal only after simultaneous addition of adsorbed bovine plasma and normal serum or after addition of normal plasma

Deficiency of the AHF plus freezing/serum factor was demonstrated in 18 patients In 12 of these the first abnormal bleeding occurred within the first year of life In a single patient the first sign of a haemorrhagic diathesis did not occur till the age of 10 years

They had all had bleedings into the skin and joints All except three fairly young patients had permanent joint deformities (see also the table fig 46) *Six declared that the disease had grown milder with increasing years* Only two adults were fully fit while four were partially fit and four were totally disabled

The recalcification times ranged between 4 min 30 sec and 20 min In 11 samples it was over 10 min

In 17 plasma samples the thrombin generation was vvery poor In one higt thrombin concentrations were obtained but after a greatly prolonged lag period

Addition of adsorbed bovine plasma alone gave a fall of the recalcification time and only slight improvement of the thrombin generation A repeated test after the patient s plasma had been stored at –20° C showed the thrombin generation to have become normal The same effect was obtained by simultaneous addition of adsorbed bovine plasma and heated readsorbed serum to fresh plasma from the patient

The literature further contains descriptions of families with a haemorrhagic diathesis (in females and males) comprising impaired capillary resistance and a reduced AHF concentration in the blood These conditions also called Wille brand Jurgens disease have nothing to do with haemophilia Christmas defect associated with impaired capillary resistance has likewise been demonstrated in females

True haemophilia in females has not been observed in this country

Some disagreement still prevails as to whether a clotting defect is present in the haemophilic transmitters Papers are continually being published however from which it seems to appear that the thromboplastic activity may be impaired in transmitters

The author has classified 18 transmitters on the basis of the clotting defects of their sons Nine out of 10 mothers in the AHF group had a prolonged clotting time as had also three out of three in the Christmas group and two mothers of patients with double defect Of two mothers of Hageman patients one had a prolonged clotting time and the other a normal clotting time The mother of a PTA patient had no clotting defect

The author submitted four transmitters to thrombin generation test In one mother of a Christmas patient the thrombin generation was poor An impaired thromboplastic activity has not been demonstrated so far in non transmitters from haemophilic families

In all the families here reported having haemophilia in two or more genera tions the disease is inherited as a sex linked recessive character irrespective of the nature of the clotting defect

Sporadic haemophilia was demonstrated in 25 families All the clotting defects except that of PTA deficiency were represented in this group Sporadic haemophilia differs neither clinically haematologically nor genetically from other forms of haemophilia Hence there is no reason to maintain this designa tion It is no independent disease

In ten families haemophilia was demonstrated in a single sibship only All the clotting defects except deficiency of the Hageman factor were represented in this group

In seven families two clotting defects were demonstrated within the same family (see the table fig 73) This phenomenon has been described previously in the literature As it has been stated in the literature that each clotting defect is due to a separate pathogenic gene the seven Danish families were investigated from this aspect On the basis of the results of this investigation the author advances the hypothesis that the various haemophilic diseases are due to a single pathogenic gene the manifestation of which varies presumably owing to the presence of different modifying genes in the individual patients This view is further borne out by other facts The disease may vary considerably in manifestation within the same family despite occurrence of only one clot ting defect The clotting defect may vary in the same patient Multiple clotting

to increase in the stated order Correspondingly the working capacity within the groups was found to rise in this order

In several instances repeated thrombin generation tests on plasma from the same patient revealed *variations in the thromboplastic activity (Chapter X)* These variations were always attended by parallel fluctuations of the recalcification time The conclusion is drawn that the variations are of plasmatic origin and not due to admixture of tissue thromboplastin during the blood sampling

Thrombocyte counts in normal and haemophilic plasma samples showed a significant difference between these, the highest figure having been obtained in the latter group *(Chapter XI)*

The symptomatic treatment of haemophilia is described in *Chapter XII* No curative treatment exists

The general treatment aims at restoring the coagulability of the blood by introducing plasma/blood or plasma fractions

A transfused antihaemophilic factor is eliminated within a few hours On the basis of studies of the literature it is recommended to give bleeding haemophilic patients *(AHF deficiency)* fresh citrated plasma in an amount corresponding to 20 per cent of the patient s blood volume and to continue with the same amount each day distributed over four daily transfusions up to 24 hours after haemostasis has been obtained

The disadvantages of transfusion are discussed especially the development of inhibitors

Treatment with AHF preparations is mentioned

It is stated in the literature that transfused *Christmas factor* preserves its activity in the organism for up to 3 weeks The author was unable to confirm this but found that we cannot reckon the effect of transfused Christmas factor (plasma or serum) to last more than 12 to 24 hours

In cases of *PTA deficiency* the clotting time is stated to remain normal for 7–10 days after transfusion of serum or plasma

Transfused *Hageman factor* (plasma or serum) is claimed to preserve its effect in the organism for less than 8 hours The author has observed bleeding in a transfused Hageman patient at a time when the thrombin generation showed normal conditions

Exchange transfusion may be necessary in haemophilic patients having inhibitors in the blood ACTH and cortisone do not improve the coagulation system *in these patients* Patients with inhibitors are in a very bad way during bleeding episodes

Chapter XIII deals with the female members of the haemophilic families

Females with true haemophilia have been described in the literature In these the defective gene occurs in homozygous form

By mating male haemophilic dogs with unquestionable *transmitters* it was possible to breed female dogs with haemophilia *(Brinkhous et al)*

PEDIGREES AND CASE REPORTS

defects are chiefly found in families having the largest number of haemophilic members The consequences of this hypothesis are discussed

Finally, mention is made of various problems within coagulation research which ought to be submitted to further investigation as well as the possibility of existence of other pathogenic factors in haemophilia, especially a thromboplastin inhibitor in the tissues of haemophiliacs

Chapter XV

PEDIGREES AND CASE REPORTS

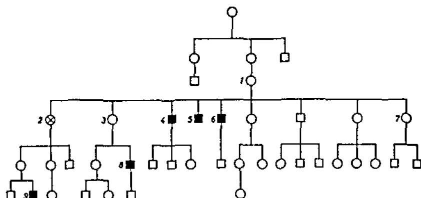
In this chapter will be rendered pedigrees of Danish haemophilic families and case reports of the patients investigated The first 35 families (up to and including family no 69) originate from *Andreassen* s work (1943) *Andreassen* s numbering of families has been preserved and continued for the subsequently collected families *Andreassen* s numbering of the individual family members up to and including family 69 has likewise been preserved Those interested are referred to *Andreassen* s work for further information on these patients

The present work includes only the case reports of the patients investigated by the author These reports are followed by statements of the results of blood analyses performed partly in the hospitals where the patients have stayed and partly by the author

The results of the thrombin generation tests are rendered graphically just as in the preceding chapters Ascissa Reaction time(T) in minutes Ordinate The reciprocal value of the fibrinogen clotting time expressed as 600/t (t in seconds)

The symbols used in the pedigrees are explained in fig 86

Family I

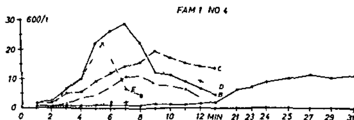


The family immigrated from Germany about 1850 As far as we know no instances of haemophilia had occurred previously in the family

4 Born 1909 1 year old the patient had prolonged oozing bleeding from an incised abscess in the forehead During childhood he had numerous oozing gingival haemorrhages which often necessitated admission to hospital There were also frequent episodes of epistaxis From the age of 7–8 he was increasingly troubled by haemarthroses in the knees elbows and ankles Between the ages of 9 and 17 the patient had to move about in a wheeled chair owing to contracture position of the knees Gradually permanent changes of contour and limited mobility also developed in the elbows and ankles He has had several episodes of painful haematuria and a few episodes of intra abdominal haemorrhage The last few years the patient has been suffering from dyspepsia due to ulcer with repeated haematemeses The disease has shown no tendency to become milder with increasing years In spite of long periods of illness the patient has been able to support himself and his family as a works manager but he eventually had to give up his job mainly owing to difficulty of walking The patient has received over 200 transfusions

Blood analyses (The Rigshospitalet (University Hospital Copenhagen) Dept A) Clotting time 1955 64 min Thrombocytes 227 000 Fibrinogen 0 35 % Serum protein 5 87 % (following haematemesis) Prothrombin 130 %

Present investigations Quick s prothrombin time 21 21 21 sec (control 20 sec) Prothrombin proconvertin according to Owren 110 % Recalcification time in dilute plasma 16 min 30 sec The thrombin generation test showed very little thrombin formation the first 13 minutes Then the thrombin concentration began to rise very slowly but it never reached high values (curve A)



Curve B Addition of 0 3 ml of normal plasma The thrombin generation normal recalcification time 3 min 30 sec

Curve C Addition of 0 3 ml of adsorbed bovine plasma Slow rise and fall of the thrombin concentration Recalcification time 3 min 25 sec

normal man

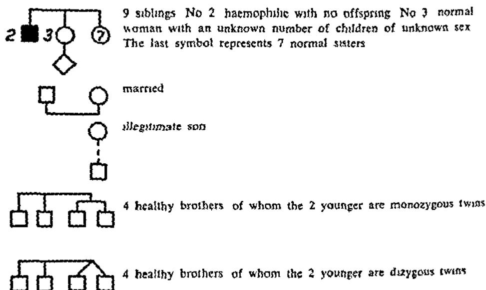
5 haemophiliac The *figure indicates the case no*

normal woman The line under the symbol indicates that she is childless

transmitter

woman with haematological evidence of heterozygosity

siblings of unknown sex The figure inside the symbol indicates the number of siblings



9 siblings No 2 haemophilic with no offspring No 3 normal woman with an unknown number of children of unknown sex The last symbol represents 7 normal sisters

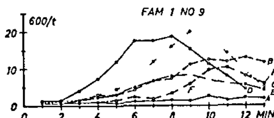
married

illegitimate son

4 healthy brothers of whom the 2 younger are monozygous twins

4 healthy brothers of whom the 2 younger are dizygous twins

Fig 86



Curve B Addition of 0 2 ml of adsorbed bovine plasma Little improvement of the thrombin generation Recalcification time 4 min 45 sec

Curve C Addition of normal serum The thrombin generation essentially improved but the lag period was long Recalcification time 4 min 45 sec

Curve D Addition of both 0 ? ml of serum and 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 35 sec

Curve E The result of the thrombin generation test after simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 2 ml of readsorbed normal plasma Recalcification time 3 min 20 sec The thrombin generation very poor

Curve F Thrombin generation test on frozen platelet rich plasma The thrombin generation poor recalcification time 5 min 30 sec

Diagnosis Deficiency of the antihaemophilic factor plus Christmas factor (combined haemophilia)

Family 2

5 Born 189? Not examined

11 Born 19 0 The patient has been liable to abnormal bleedings since the age of 6 months in infancy chiefly oozing gingival bleedings and epistaxis as well as large often spontaneous subcutaneous haematomas on the body Since the age of 3 years there has been an increasing tendency to bleedings in the knees elbows wrists and ankles Permanent changes have gradually developed in the knees and elbows causing limited mobility He has had frequent intramuscular bleedings but never neurological pressure symptoms Since school age he has had several episodes of haematuria No other intra abdominal haemorrhages have been observed The disease does not seem to have assumed a milder character in the course of years The patient is periodically unfit for work and receives permanent sickness benefit He seems to be the most severely affected haemo philiac in the family He has received numerous blood transfusions usually with good effect

Blood analyses 1937 (The Rigshospitalet Medical Out Patient Department) Clotting time Howell Gram 10 min thrombocytes 226 000 bleeding time 5 min

194. *(And eassen)* Clotting time 1) (slow venous puncture) 8 /₂–11 min Clot firm 2) (normal venous puncture) 21¹/₂–31 min Clot loose Blood platelets 263 000 E S R 2 min Blood type A

Present investigations Quick s prothrombin time 17 18 18 sec (control 18 sec) Prothrombin proconvertin according to Owren 86 % Recalcification time in dilute plasma 7 min 40 sec The thrombin generation test gave very low concentrations of thrombin (curve A) the first 7 min Then followed a slow rise of the concentration which culminated after 13 min thereafter to fall rather steeply

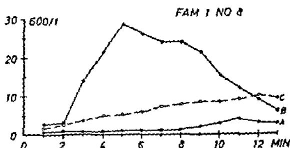
Curve D Addition of 0 2 ml of normal serum The thrombin generation somewhat improved after a lag period of 8 minutes Recalcification time 4 min 30 sec
Curve E Simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 2 ml of normal serum The thrombin generation normal Recalcification time 2 min 20 sec
Curve F When adsorbed serum was substituted for normal serum the thrombin generation improved but little

Diagnosis Deficiency of the antihaemophilic factor plus Christmas factor (combined haemophilia)

8 Born 1926 The first abnormal bleeding occurred at the age of 3 years In infancy the patient had frequent oozing gingival haemorrhages of several days duration No epistaxis From the age of 5–6 he had repeated haemarthroses especially in the knees and ankles as well as the left hip joint He has fractured the shaft of the left femur twice without this having caused much complicating haemorrhage The fractures healed up in normal position There have been a few episodes of renal haemorrhage the latest one in *1956 The patient presents no permanent joint deformities except that the left knee* can be bent only from 180 to 90 degrees (a sequel of fracture) There have never been gastro intestinal haemorrhages nor spontaneous subcutaneous bleeding

The patient was sterilised in 1950 There was a little oozing haemorrhage following the operation and a haematoma developed in the operation wound but no other complications He has been given a single transfusion On one occasion he received an injection of antihaemophilic factor (The State Serum Institute Copenhagen) owing to haematuria with good effect The patient felt greatly handicapped by the disease in boyhood but since 1946 it has caused him little trouble The patient is fit to work as a technical assistant

Blood analyses Quick s prothrombin time 20 20 23 sec (control 20 sec) Thrombocytes 358 000 per µl of plasma Recalcification time in dilute plasma 12 min The *thrombin generation test showed practically no thrombin generation (curve A)*



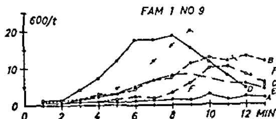
Curve B Addition of 0 3 ml of adsorbed bovine plasma The thrombin generation normal *the recalcification time fell to 2 min*
Curve C Addition of 0 2 ml of normal serum The thrombin generation very slightly improved Recalcification time 4 min

Diagnosis Deficiency *of the antihaemophilic factor (classical haemophilia)*

9 Born 1950 The patient has since birth been liable to bruises 1 year old he had several prolonged bleedings after having bitten his tongue or lip During the period of crawling large subcutaneous haematomas occurred on the buttocks and scrotum

Blood analyses 1951 (The Children s Hospital Martinsvej) Thrombocytes 287 000

Present investigations Prothrombin time 19 19 19 sec (control 19 sec) Thrombocytes 466 000 per µl plasma Clotting time according to Burker Andreassen 8 → 66 min Recalcification time in dilute plasma 15 min The thrombin generation test showed hardly any thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma Little improvement of the thrombin generation Recalcification time 4 min 45 sec
Curve C Addition of normal serum The thrombin generation essentially improved but the lag period was long Recalcification time 4 min 45 sec
Curve D Addition of both 0 ? ml of serum and 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 35 sec
Curve E The result of the thrombin generation test after simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 ? ml of readsorbed normal plasma Recalcification time 3 min ?0 sec The thrombin generation very poor
Curve F Thrombin generation test on frozen platelet rich plasma The thrombin generation poor recalcification time 5 min 30 sec

Diagnosis Deficiency of the antihaemophilic factor plus Christmas factor (combined haemophilia)

Family 2

5 Born 189? Not examined

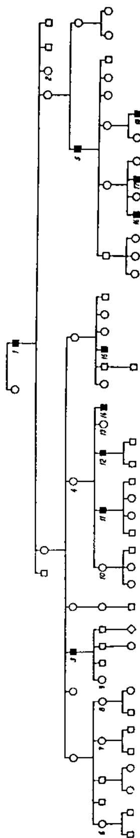
11 Born 1920 The patient has been liable to abnormal bleedings since the age of 6 months in infancy chiefly oozing gingival bleedings and epistaxis as well as large often spontaneous subcutaneous haematomas on the body Since the age of 3 years there has been an increasing tendency to bleedings in the knees elbows wrists and ankles Permanent changes have gradually developed in the knees and elbows causing limited mobility He has had frequent intramuscular bleedings but never neurological pressure symptoms Since school age he has had several episodes of haematuria No other intra abdominal haemorrhages have been observed The disease does not seem to have assumed a milder character in the course of years The patient is periodically unfit for work and receives permanent sickness benefit He seems to be the most severely affected haemo philiac in the family He has received numerous blood transfusions usually with good effect

Blood analyses 1937 (The Rigshospitalet Medical Out Patient Department) Clotting time Howell-Gram 10 min thrombocytes 226 000 bleeding time 5 min

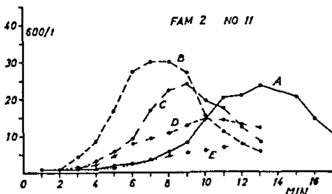
1942 *(Andreassen)* Clotting time 1) (slow venous puncture) 8½–11 min Clot firm 2) (normal venous puncture) 21 ½–31 min Clot loose Blood platelets 263 000 E S R 2 min Blood type A

Present investigations Quick s prothrombin time 17 18 18 sec (control 18 sec) Prothrombin proconvertin according to Owren 86 % Recalcification time in dilute plasma 7 min 40 sec The thrombin generation test gave very low concentrations of thrombin (curve A) the first 7 min *Then followed a slow rise of the concentration which* culminated after 13 min thereafter to fall rather steeply

Family 2



5 Born 1892 Not examined



Curve B Addition of 0 2 ml of ad orbed bovine plasma The thrombin generation nor mal recalcification time 3 min
Curve C Addition of 0 2 ml of normal serum The thrombin generation nearly nor mal though slightly delayed recalcification time 4 min 25 sec
Curve D Platelet rich plasma after storage at — 0 for 24 hours The thrombin genera tion poor recalcification time 4 min 45 sec
Curve E Platelet rich plasma after storage at 0 C for 24 hours The thrombin generation poorer than in the fresh plasma Recalcification time 8 min 10 sec

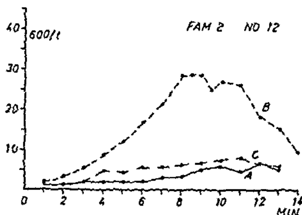
Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)
(On previous testing plasma from the above patient had shown even poorer thrombin generation A normal thrombin generation was not obtained then by adding plasma from a Christmas patient or plasma from an AHF patient but only after simultaneous addition of such two plasma samples The patient s clotting defect thus seems to vary The experiment described above (curve C) also seems to suggest a combined defect)

12 Born 1922 A bleeding tendency has been present from the first year of life In infancy the patient had frequent episodes of prolonged oozing epistaxis and gingival haemorrhage which often necessitated admission to hospital He also had frequent sub cutaneous haematomas on the body which often seemed to develop spontaneously Since school age there was an increasing tendency to bleeding in the large joints chiefly the knees and elbows The contours of these joints have gradually changed and the mobility is somewhat limited He has had several episodes of haematuria and also many intra muscular bleedings The last few years the patient has often had melaena with associated symptoms of severe anaemia The disease has not grown milder in the course of years The patient receives permanent sickness benefit but is able to do light work as an artisan thereby supplementing his income He has received several transfusions with good effects

Blood analyses 1937 (The Rigshospitalet Medical Out Patient Dept) Clotting time (direct) 15 min Thrombocytes 3₂6 000 Bleeding time 8 min

1942 *(Andreassen)* Clotting time 19[1] –27[1/2] min Clot loose Thrombocytes 357 000 E S R 4 min

Present investigations Clotting time according to Burker Andreassen 12–25½ min Spontaneous clotting time at room temp 50 min Quick s prothrombin time 17 17 17 sec (control 18 sec) Prothrombin proconvertin according to Owren 100 % Recalcification time in dilute plasma 10 min The thrombin generation test showed a very poor thrombin generation (Curve A)



Curve B Addit on of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 1 min 30 sec

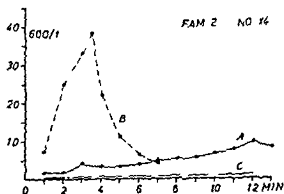
Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 4 min 45 sec

Diagnosis Defi iency of the antihaemophilic factor (classical haemophilia)

14 Born 1931 The first abnormal bleeding occurred at the age of 3–4 months In infan y the patient had frequent prolonged oozing gingival haemorrhages and frequent most often spontaneous subcutaneous haematomas From the age of 2–3 years there were in reasingly frequent usually spontaneous painful haemarthroses especially in the elbows and knees which have resulted in permanently limited mobility Repeated bleedings in the proximal phalanx of the left big toe has resulted in plantar flexion of the toe The patient therefore has to wear an orthopaedic boot There have been several episodes of intramuscular haemorrhage without symptoms of compression Haematuria or intra abdominal haemorrhages have not been observed The patient receives permanent sickness benefit He seems to be less troubled by the disease than his brothers He has a supplementary income by some light artisan s work

Blood analyses 1951 Clotting time according to Burker Andreassen 8½–22½ min.

Present investigations Quick s prothrombin time normal Prothrombin proconvertin according to Owren 87 % Recalcification time in dilute plasma 5 min The thrombin generation test showed very slight thrombin generation (*Curve A*)



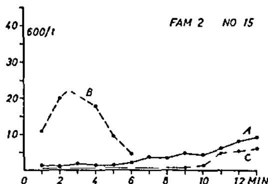
Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin gener tion normal Recalcification time 1 min 30 sec

Curve C Addition of 0 2 ml of serum The thrombin generation poor Recalcification time 16 min 30 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

15 Born 1934 A bleeding tendency with subcutaneous haematomas was present from the age of 1 year There have been frequent bleedings from the gums and nose Later there occurred increasingly frequent haemarthroses comprising especially the kn es elbows and ankles In the knees these resulted in limited mobility Since the age of 15 he has occasionally had haematuria but never melaena nor haematemesis There was prolonged bleeding from minor cuts and on secondary dentition The patient is under public care of mental defectives

Blood analyses Clotting time (Burker Andreassen) 8–23 min Quick s prothrombin time 23 25 23 sec (control 21 sec) Prothrombin proconvertin according to Owren 78 % Recalcification time in dilute plasma 9 min The thrombin generation test showed very little thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcifi ation time 1 min

Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 10 min 30 sec

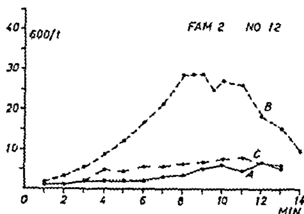
Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

16 Born 1938 A bleeding tendency was first noticed when the patient was about 1 year old It manifested itself by extravasation of blood into the left knee He has since had repeated apparently spontaneous bleedings into the left knee Since 1949 there have been permanent radiographic changes with halisteresis and irregular articular surfaces in this knee as well as limited mobility The patient bruises easily and has prolonged oozing bleeding from cuts The parents state that the patient is less troubled by the disease than his brother (no 17)

Blood analyses 1946 (The Rigshospitalet Paediatric Dept) Thrombocytes 286 000 Clotting time 10–12 min Gothlin 10 petechiae Bleeding time 15 min

1947 (The Rigshospitalet Paediatric Dept) Thrombocytes 310 000 Clotting time 47 min and 45 min Bleeding time 1½ min Gøthlin 5 petechiae

P esent investigations Quick s prothrombin time 15 16 17 sec (control 20 sec) Recalcification time in dilute plasma 6 min The thrombin generation test showed a very slow rise of the thrombin concentration whi h reached high values after 12 min (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 1 min 30 sec

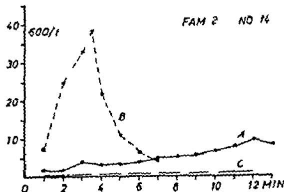
Curve C Addition of 0 2 ml of *normal serum The thrombin generation poor* Recalcification time 4 min 45 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

14 Born 1931 *The first abnormal bleeding occurred* at the age of 3–4 months In infancy the patient had frequent prolonged oozing gingival haemorrhages and frequent *most often spontaneous* subcutaneous *haematomas From the age of 2–3 years there were* increasingly frequent usually spontaneous painful haemarthroses especially in the elbows and knees which have resulted in permanently limited mobility Repeated bleedings in the proximal phalanx of the left big toe has resulted in plantar flexion of the toe The patient therefore has to wear an orthopaedic boot There have been several episodes of *intramuscular haemorrhage without symptoms of compression Haematuria* or intra abdominal haemorrhages have not been observed The patient receives permanent sickness benefit *He seems to be less troubled by the disease than his brothers He has a* supplementary income by some light artisan s work

Blood analyses 1951 Clotting time according to Burker Andreassen $8\frac{1}{2}$–$22\frac{1}{2}$ min

Present investigations Quick s prothrombin time normal Prothrombin proconvertin according to Owren 87 % Recalcification time in dilute plasma 5 min The thrombin *generation test showed very slight thrombin generation (Curve A)*



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 1 min 30 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation unchanged poor Recalcification time 6 min

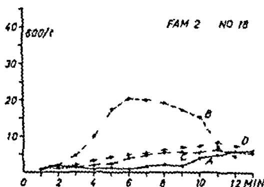
Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

18 Born 1947 At the age of 10 months the patient had a posttraumatic haemorrhage from the frenulum of the upper lip He had frequent prolonged bleedings from the lips and tongue He has had several subcutaneous and intramuscular haematomas There have been numerous bleedings in the knees shoulders elbows and ankles but these have not caused limited mobility of the joints He has never had haematuria melaena or haematemesis The patient has been given several transfusions

Blood analyses (The Rigshospitalet Paediatric Dept) Thrombocytes 344 000 Prothrombin time normal Clotting time beginning 11¼ min concluded 12¾ min Bleeding time *indefinite Capillary resistance normal Plasma fibrinogen* 0 3 % Osmotic resistance normal Serum calcium 9 7–9 9 mg %

1948 (Med Dept County Hospital Næstved) Thrombocytes 275 000 Bleeding time 6 *min* Clotting time 8 min Prothrombin time 91

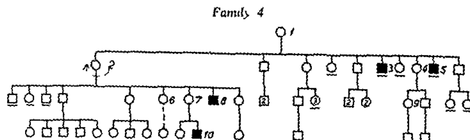
Present investigations 1956 Quick's prothrombin time 21 21 21 sec (control 19 sec) Recalcification time in dilute plasma 10 min 45 sec The thrombin generation showed hardly any thrombin generation (Curve A)

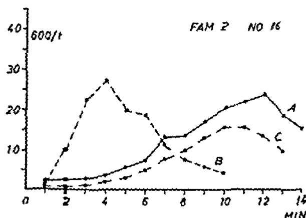


Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 45 sec
Curve C Addition of 0 ml of normal serum The thrombin generation poor Recalcification time 6 min 30 sec
Curve D Addition of platelet suspension No improvement of the thrombin generation

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 4





Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min
Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 5 min 25 sec

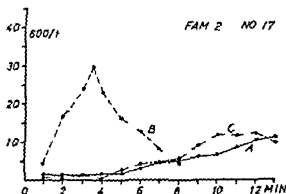
Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

17 Born 1944 The patient has bruised easily since babyhood At the age of 2 years he bled for 13 days from a small cut At the age of 3 he had a posttraumatic bleeding from the gum which continued for 9 days despite three transfusions and tamponade He has repeatedly been in hospital with bleedings in the knees ankles and elbows The right knee presents an extension defect of 10 degrees The bones of the ankles are slightly halisteretic In 1954 a sublingual haematoma occurred which caused difficulty of swallowing

Blood analyses 1947 (The Rigshospitalet Paediatric Dept) Thrombocytes 166 000 Prothrombin time (Larsen & Plum) 70 sec (control 22 sec)

1954 (The Rigshospitalet Paediatric Dept) Thrombocytes 180 000 Prothrombin time 28 sec (control 18 sec) Bleeding time 35 min Capillary resistance 3 petechiae Clotting time 205 min (beginning) – 335 min (complete) Osmotic resistance 0 52/0 38 (control 0 46/0 36)

Present investigations Quick s prothrombin time 19 19 19 sec (control 19 sec) Recalcification time in dilute plasma 7 min 40 sec The thrombin generation test showed very little thrombin generation (Curve A)



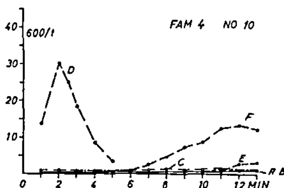
Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 1 min 45 sec

10 Born 1946 Few months old the patient bled for a long time from a sty Since the age of 4 years there have been repeated bleedings specially in the knees and elbows as a rule following slight injuries There is intermittent greatly reduced mobility of the knee joints At the age of 5 he had a bleeding in the left wrist These bleedings have left no permanent changes He has had a few episodes of prolonged epistaxis and prolonged bleeding after sampling from the ear The patient has been given several transfusions

Blood analyses 1951 (Dronning Louises Children s Hospital) Clotting time according to Howell Gram 20 → 30 min Bleeding time 2 min. Capillary resistance 80 mm Hg for 5 min No petechiae Thrombocytes 376 000 Prothrombin time normal Fibrinogen 0 3 % Serum calcium 91 mg % Serum protein 7 3 % Serum albumin 5 2 % Serum globulin 2 1 %

1955 (Dronning Louises Children s Hospital) Thrombocytes 215 000 Bleeding time indefinite Clotting time 10½-13 min 30 min 9-15 min Howell Gram > 30 min Prothrombin time 28 sec (control 29 sec) Capillary resistance No petechiae

Present investigations Clotting time (Burker Andreassen) 6 min → 30 min Quick s prothrombin time 21 21 21 sec (control 20 sec) Recalcification time in dilute plasma 19 min 54 sec The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 5 ml of frozen normal platelet suspension (142 000 platelets per cubic millimetre of suspension) No improvement of the thrombin generation Recalcification time 9 min 42 sec

Curve C Platelet rich citrated plasma stored 24 hours at −20 C The thrombin generation poor though the clotting times in the fibrinogen tubes was somewhat shorter Recalcification time 5 min 50 sec Freezing of platelet poor plasma gave no visible improvement either of the thrombin generation Recalcification time 12 min 15 sec

Curve D Addition of normal serum The thrombin generation normal Recalcification time 1 min 35 sec

Curve E Heated readsorbed serum No improvement of the thrombin generation Recalfication time 10 min 20 sec

Curve F Addition of 0 2 ml of adsorbed bovine plasma Little improvement of the thrombin generation after a lag period of 6 min Re alcification time 6 min 50 sec

Diagnosis Christmas factor deficiency (Christmas disease)

The family cannot be traced further back than to no 1 who did not know her parents having been placed out among non relatives in early infancy

3 Born 1890 Propositus A bleeding tendency was observed from the patient's first year of life In infancy mucosal haemorrhages from the nose and gums predominated Since school age there have been increasingly frequent haemarthroses comprising nearly all the large joints chiefly the knees and elbows in which permanent changes have gradually developed X ray of the knee joints (The Rigshospitalet Dept D 1937) revealed bony atrophy and deformities of the joint surfaces due to the presence of several *osteophytes He has had several episodes of renal haemorrhage* but no other intra abdominal bleedings There have been numerous intramuscular bleedings which have left no permanent neurological symptoms The disease has been steadily progressive since 1951 In 1955 he was bedridden for 6 months owing to profuse bleeding in the left shoulder That same year he suddenly became hoarse with associated difficulty of breathing He was intubated and admitted to an otologic unit where a large haematoma was detected in the left piriform recess and a smaller one in the right arytenoid region After extubation the respiration was unimpeded again

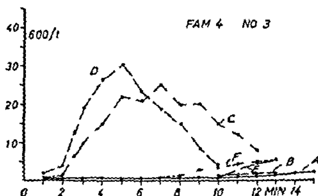
The movements of the right shoulder joint and both knee joints have become greatly limited The left knee is fixed in 30 degrees flexion and the right can be bent 15 degrees The right hip is almost ankylotic The patient who is totally disabled has received several *transfusions Once during an episode of haematuria he was given antihaemophilic globulin* with no effect whatever

Blood analyses 1924 (The Rigshospitalet Dept B) Clotting time according to Howell Gram 12 min Bleeding time 3¹/ min Thrombocytes 248 000

1940 (The Rigshospitalet Dept D) Howell Gram 19 min Thrombocytes 376 000

1955 (The Rigshospitalet Dept B) Prothrombin 92¹/₂ % Prothrombin in serum 108 % Thrombocytes 247 000 Clotting time 3¹/₄–5 min Urine microscopy numerous erythrocytes Blood urea 29 mg %

Present investigations Prothrombin proconvertin (Owren) 115 % Recalcification time in dilute plasma 12 min The thrombin generation test showed hardly any thrombin generation within 15 min (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma No improvement of the thrombin generation *Recalcification time 12 min*

Curve C Addition of 0 2 ml of citrated plasma from a patient with classical haemophilia (fam 68 pt no 1) The thrombin generation normal Recalcification time 3 min

Curve D Addition of *0 2 ml of normal serum The thrombin generation normal* recalcification time 2 min

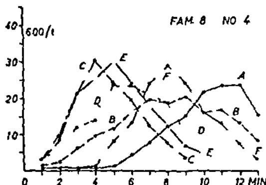
Curve E Addition of 0 2 ml of heated readsorbed serum No essential improvement of the thrombin generation *Recalcification time 10 min*

Curve F A similar result was obtained after addition of 0 2 ml of normal frozen platelet suspension (178 000 platelets c mm)

Diagnosis Christmas factor deficiency (Christmas disease)

4 Born 1906 A bleeding tendency especially from the gums and nose has been present since infancy There has always been prolonged bleeding after dental extraction The patient has had several subcutaneous haematomas often so extensive and painful as to require admission to hospital He has had several intramuscular bleedings following injuries but no abnormal bleeding from cuts Haemarthroses have never been observed Apart from a single episode of haematuria the patient has had no visceral haemorrhages The disease has grown milder with increasing years especially since the age of 30 though he is still liable to intramuscular haemorrhages and ecchymoses after injuries The patient is a farmer and is able to support himself and his family

Blood analyses Quick s prothrombin time 21 21 21 sec (control 20 sec) Thrombocytes 1 9 000 per μl of plasma Recalcification time in dilute plasma 6 min 45 sec The thrombin generation test showed fairly quick thrombin generation after a lag period of 5 min The thrombin concentration reached rather high values (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 30 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 1 min 45 sec

Curve D Addition of 0 2 ml of heated readsorbed normal serum The thrombin generation normal Recalcification time 1 min 45 sec

Curve E The variation of the thrombin concentration after addition of 0 2 ml of fresh platelet suspension (150 000 platelets per μl of solution) The thrombin generation normal Recalcification time 1 min 30 sec

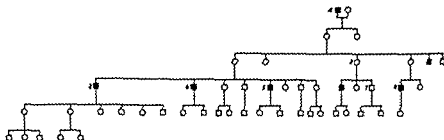
Curve F The thrombin generation normal after the plasma had been frozen down to −20 C for 18 hours with its normal content of platelets Recalcification time 4 min 45 sec

0 2 ml of the patient s plasma was *not* able to normalise the thrombin generation in plasma from a patient with pronounced Christmas factor deficiency (family 85 no 1)

Diagnosis Slight Christmas factor deficiency (Christmas disease)

5 Born 1913 The patient has since the age of 4–5 years been liable to bleedings from the gums and nose which though prolonged have rarely been severe *However* at the age of 9 the patient bled for 9 days and nights after extraction of all his teeth He had to have 13 transfusions He does not bleed abnormally from cuts and has never had haemarthroses or visceral haemorrhages At the age of 31 the patient was herniotomised He began to bleed 24 hours after the operation and continued for 6–7 days He was given several transfusions Apart from the gingival haemorrhages the patient feels that the disease has improved appreciably since the age of 30 He manages his work as a farmer without difficulty

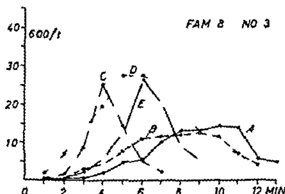
Family 8



The family originates from a small island where it can be traced far back The patient marked A who was a fiddler is believed to have suffered from a mild degree of haemophilia He attained the age of 81

3 Born 1904 A bleeding tendency was present from the age of 6–7 years manifesting itself chiefly by prolonged oozing haemorrhages from the gums and nose Also he was liable to subcutaneous haematomas partly spontaneous and partly after minor injuries Haemarthroses have never been observed The patient has once had haematuria but never other visceral haemorrhages At the age of 44 the patient had all his teeth extracted He then bled for 9 days and nights and had to have nine transfusions There is no abnormal bleeding after cuts Apart from the above prolonged gingival haemorrhages bleedings have become rare since the age of about 30 and the patient is fully fit for his work as a farmer

Blood analyses Quick s prothrombin time 15 17 16 sec (control 17 sec) Thrombo cytes 203 000 per μl of plasma Recalcification time in dilute plasma 4 min 10 sec The thrombin generation test showed a slow rise of the thrombin concentration which did not reach particularly high values (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma Some acceleration of the throm bin generation Recalcification time 3 min 21 sec

Curve C Addition of 0 2 ml of the patient s own serum after this had been stored at −20 C for one year The thrombin generation normal Recalcification time 3 min

Curve D *Addition of heated readsorbed serum The thrombin generation normal* Re calcification time 2 min

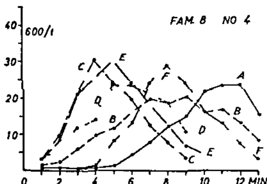
Curve E After storage of the patient s plasma for 24 hours at −20 C the thrombin generation was normal Recalcification time 3 min 50 sec

The patient s plasma normalised the thrombin generation in plasma from patients with Christmas disease (fam 4 no 10 and fam 85 no 1)

Diagnosis *Presumably the Hageman trait*

4 Born 1906 A bleeding tendency especially from the gums and nose has been present since infancy There has always been prolonged bleeding after dental extraction The patient has had several subcutaneous haematomas often so extensive and painful as to require admission to hospital He has had several intramuscular bleedings following injuries but no abnormal bleeding from cuts Haemarthroses have never been observed Apart from a single episode of haematuria the patient has had no visceral haemorrhages The disease has grown milder with increasing years especially since the age of 30 though he is still liable to intramuscular haemorrhages and ecchymoses after injuries The patient is a farmer and is able to support himself and his family

Blood analyses Quick s prothrombin time 21 21 21 sec (control 20 sec) Thrombo cytes 1 9 000 per µl of plasma Recalcification time in dilute plasma 6 min 45 sec The thrombin generation test showed fairly quick thrombin generation after a lag period of 5 min The thrombin concentration reached rather high values (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation nor mal Recalcification time 2 min 30 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Re calcification time 1 min 45 sec

Curve D Addition of 0 ² ml of heated readsorbed normal serum The thrombin genera tion normal Recalcification time 1 min 45 sec

Curve E The variation of the thrombin concentration after addition of 0 2 ml of fresh platelet suspension (150 000 platelets per µl of solution) The thrombin generation nor mal Recalcification time 1 min 30 sec

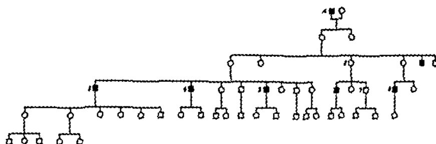
Curve F The thrombin generation normal after the plasma had been frozen down to −20 C for 18 hours with its normal content of platelets Recalcification time 4 min 45 sec

0 2 ml of the patient s plasma was *not* able to normalise the thrombin generation in plasma from a patient with pronounced Christmas factor deficiency (family 85 no 1)

Diagnosis Slight Christmas factor deficiency (Christmas disease)

5 Born 1913 The patient has since the age of 4–5 years been liable to bleedings from the gums and nose which though prolonged have rarely been severe However at the age of 29 the patient bled for 9 days and nights after extraction of all his teeth He had to have 13 transfusions He does not bleed abnormally from cuts and has never had haemarthroses or visceral haemorrhages At the age of 31 the patient was herniotomised He began to bleed 24 hours after the operation and continued for 6–7 days He was given several transfusions Apart from the gingival haemorrhages the patient feels that the disease has improved appreciably since the age of 30 He manages his work as a farmer without difficulty

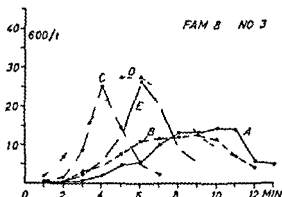
Family 8



The family originates from a small island where it can be traced far back The patient marked 4 who was a fiddler is believed to have suffered from a mild degree of haemophilia He attained the age of 81

3 Born 1904 A bleeding tendency was present from the age of 6–7 years manifesting itself chiefly by prolonged oozing haemorrhages from the gums and nose Also he was liable to subcutaneous haematomas partly spontaneous and partly after minor injuries Haemarthroses have never been observed The patient has once had haematuria but never other visceral haemorrhages At the age of 44 the patient had all his teeth extracted He then bled for 9 days and nights and had to have nine transfusions There is no abnormal bleeding after cuts Apart from the above prolonged gingival haemorrhages bleedings have become rare since the age of about 30 and the patient is fully fit for his work as a farmer

Blood analyses Quick s prothrombin time 15 17 16 sec (control 17 sec) Thrombocytes 203 000 per μl of plasma Recalcification time in dilute plasma 4 min. 10 sec The thrombin generation test showed a slow rise of the thrombin concentration which did not reach particularly high values (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma Some acceleration of the thrombin generation Recalcification time 3 min 21 sec

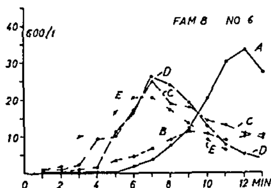
Curve C Addition of 0 2 ml of the patient s own serum after this had been stored at –20 C for one year The thrombin generation normal Recalcification time 3 min

Curve D Addition of heated readsorbed serum The thrombin generation normal Recalcification time 2 min

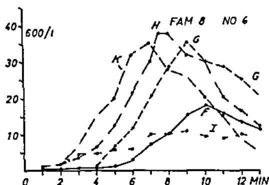
Curve E After storage of the patient s plasma for 24 hours at –20 C the thrombin generation was normal Recalcification time 3 min 50 sec

The patient s plasma normalised the thrombin generation in plasma from patients with Christmas disease (fam 4 no 10 and fam 85 no 1)

Diagnosis Presumably the Hageman trait



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poorer The recalcification time fell however to 4 min 45 sec
Curve C Addition of 0 2 ml of normal plasma The thrombin generation normal Recalfication time 3 min 45 sec
Curve D Addition of adsorbed normal serum The thrombin generation normal Recalfication time 4 min 30 sec
Curve E Addition of 0 2 ml of the patient s own serum The thrombin generation normal Recal ification time 3 min 17 sec



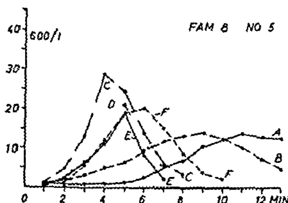
Curve F Addition of 0 2 ml of heated readsorbed serum No improvement of the thrombin generation Recalcification time 6 min 45 sec
Curve G After the plasma had been stored with its normal platelet content at −20 C the thrombin generation was accelerated to become almost normal The recalcification time was slightly prolonged 5 min 20 sec

Two years after the above investigations the patient s blood was submitted to another series of tests owing to a suspicion that the lack of the Christmas factor was not total Quick s prothrombin time was normal The recalcification time in dilute plasma was 3 min 40 sec Thrombocytes 580 000 per µl of plasma The thrombin generation was perfectly normal (Curve H) Addition of 0 2 ml of this plasma to plasma from a patient with Christmas factor deficiency (fam 62 no 3) gave no essential improvement of the thrombin generation of the latter (Curve I) On the other hand by adding 0 4 ml to the same volume of plasma a perfectly normal thrombin generation was obtained (Curve K)

Diagnosis (Slight) Christmas factor deficiency (Christmas disease)

7 Born 1919 The patient has always bruised easily He has had a few 14 days long episodes of profuse haemorrhage following dental extraction There have never been

Blood analyses Quick s prothrombin time 18 18 18 sec (control 18 sec) Thrombocytes 112 000 per µl of plasma Recalcification time in dilute plasma. 6 min 15 sec Bexelius no petechiae The thrombin generation test showed fairly little thrombin generation (Curve A) The concentration rose somewhat however after a lag period of 5 min



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation somewhat improved but hardly normal Recalcification time 3 min 30 sec
Curve C Addition of 0 2 ml of serum The thrombin generation normal Recalcification time 2 min 30 sec
Curve D The thrombin generation normal after the patient s plasma had been stored with its normal platelet content at −20 C for 24 hours Recalcification time 2 min 45 sec
Curve E The thrombin generation also became normal after freezing of the patient s platelet poor plasma (6000 thrombocytes per µl) Recalcification time 3 min 40 sec
Curve F Addition of 0 2 ml *of heated readsorbed serum The thrombin generation* normal Recalcification time 3 min 15 sec

0 2 ml of the patient s plasma was not able to normalise the thrombin generation in plasma with pronounced Christmas factor deficiency (fam 85 no 1)

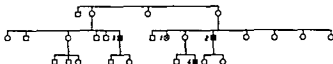
Diagnosis Slight Christmas factor deficiency (Christmas disease)

6 Born 1911 Propositus A bleeding tendency first appeared at the age of 5 or 6 years as a prolonged *oozing haemorrhage from a small wound in the back of the head* There have often since been oozing bleedings from the gums and a few episodes of heavy epistaxis Since the age of 10 the patient has had several haemarthroses most often in the knees and ankles There are no permanent joint deformities The patient has had numerous episodes of haematuria the first one at the age of 17 He has never had haematemesis nor melaena but often intramuscular haemorrhages The patient s bleeding tendency has declined since the age of 38–39 The patient has been considerably more affected than the remaining bleeders of the family Nevertheless he has been able to take a university degree and can support his family

Blood analyses 1949 (Bispebjerg Hospital Dept A) Thrombocytes 178 000 per µl of plasma Capillary resistance normal Clot retraction normal Prothrombin time 72–89 % Spontaneous clotting time at 37 C 5 min

Present investigations Quick s prothrombin time 19 19 18 sec (control 19 sec) *Recalcification* time in dilute plasma 7 min 30 sec The thrombin generation test showed very considerable thrombin concentrations after a lag period of about 7 min (Curve A)

Family 10

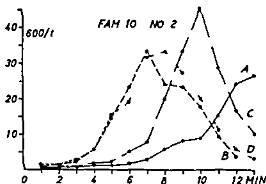


The family immigrated from Sweden about 1840

2 Born 1914 Propositus The patient has had a bleeding tendency since the age of 6 months In infancy there had been frequent prolonged oozing mucosal haemorrhages from the nose and mouth of which especially the gingival bleedings often necessitated hospitalisation From school age the patient had increasingly frequent haemarthroses gradually comprising nearly all the large joints chiefly the knees and elbows where they left permanent changes X ray of the knee joints in 1937 (The Rigshospitalet Dept C) revealed halisteresis and subchondral areas of decreased density as well as narrowed joint cavities He has had many episodes of haematuria accompanied by unilateral lumbar pain Intra abdominal haemorrhages were observed at the age of 34 There have often been intramuscular bleedings Such a bleeding in the left forearm was once accompanied by paresthesiae of the left hand These subsided in the course of 6 months Although the patient still oc asionally has haemarthroses and subcutaneous haemorrhages he feels that the disease has grown milder since the age of 30 The patient is head-clerk in a business and is able to support himself and a family

Blood analyses (Andreassen) 1934 Clotting time according to Howell Gram 10 min 30 sec Thrombocytes 891 000 1937 Clotting time 9 min 30 sec – 16 min

Present investigations Quick s prothrombin time 20 19 19 sec (control 19 sec) Thrombocytes 590 000 per µl of plasma Recal ification time in dilute plasma 8 min 22 sec The thrombin generation test showed hardly any thrombin generation the first 6 minutes after which the thrombin concentration rose slowly to reach high values (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 40 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation somewhat accelerated but the lag period still 5–6 min The inactivation occurred rather quickly Recal ification time 6 min

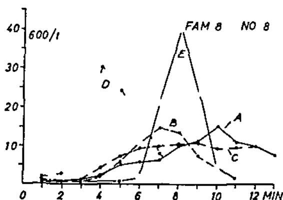
Owing to the course of curve A showing a rather steep though delayed rise of the thrombin concentration the following experiments were made as well

haemarthroses haematemesis nor melaena The patient is not troubled by his disease in his daily work

Blood analyses Quick s prothrombin time 20 22 22 sec (control 20 sec) Spontaneous clotting time for whole blood at 37 C 7 min Recalcification time in dilute plasma 4 min Thrombocytes 183 000 per µl of plasma The thrombin generation showed no definitely pathological conditions

8 Born 1927 The patient had in childhood had prolonged bleedings after having bit his lip Dentition caused no haemorrhages whereas contusions gave prolonged bleeding The patient has never had haemarthroses haematuria haematemesis nor melaena He has had several episodes of prolonged epistaxis There has been a single subcutaneous haematoma on the forearm and a haemorrhage in one wrist He has done his military service (as a dragoon) without great inconvenience A diagnostic maxillary sinus puncture entailed prolonged bleeding which stopped after transfusion The patient is fully fit for farming and is able to support his family

Blood analyses Quick s prothrombin time 19 19 19 sec (control 19 sec) Thrombocytes 179 000 per µl of plasma Recalcification time in dilute plasma 4 min 15 sec The thrombin generation test showed a very low thrombin generation The thrombin concentration reached only low values (Curve A)



Curve B The thrombin generation normal after the plasma had been frozen to −20 C for 24 hours with its normal platelet content though the maximum concentration was fairly low Recalcification time 4 min 45 sec

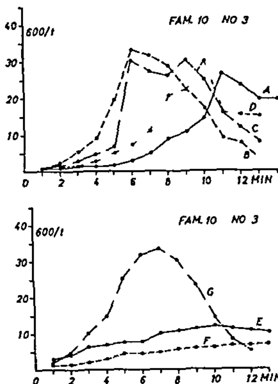
Curve C Addition of 0 2 ml of adsorbed bovine plasma No unquestionable improvement of the thrombin generation Recal ification time 3 min 50 sec

Curve D *Addition of 0 2 ml of normal serum The thrombin generation normal* Re calcification time 2 min 40 sec

Curve E Addition of 0 2 ml of readsorbed heated serum The thrombin generation de layed the lag period was 6 min Recalcification time 6 min 15 sec

0 2 ml of the patient s plasma was unable to normalise the thrombin generation in plasma with a pronounced Christmas factor deficiency (fam 85 no 1)

Diagnosis (Slight) Christmas factor deficiency (Christmas disease)



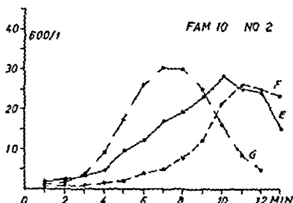
Curve E. Storage of the patient's plasma with its normal platelet content at −20 C for 24 hours gave a poorer thrombin generation At the same time the recalcification time was reduced 2 min 35 sec

Addition of 0 2 ml of the patient's plasma to plasma from another patient with AHF deficiency (fam 71 no 4) did not improve the thrombin generation in the plasma of the patient concerned (curve F) Recalcification time 6 min 45 sec After 0 2 ml of readsorbed serum had been added to the patient's plasma the thrombin generation became perfectly normal (Curve G) The recalcification time was 2 min 50 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

4 Born 1942 At the age of 1 year the patient was in hospital owing to prolonged bleeding from an injured upper lip He is liable to large subcutaneous extravasations of blood In 1948 the patient was admitted to hospital on account of a large subcutaneous haematoma over the right ligament, extending into the right flank He has never had remarkable epistaxis There have been a few episodes of haematuria but never melaena nor haematemesis From the age of 1 year he fairly often had posttraumatic haemarthroses especially in the elbows and ankles more rarely in the knees These have not yet caused permanent changes Cuts never bled extraordinarily The patient has never received a blood transfusion In addition to the bleeding tendency the patient suffers from asthma He has missed about one third of his school days on account of the haemophilia

Blood analyses Quick's prothrombin time 19 19 18 sec (control 18 sec) Thrombocytes 359 000 per μl of plasma Recalcification time in dilute plasma 7 min The thrombin generation test showed a very slow rise of the thrombin concentration which did not reach high values (Curve A)



Curve D *Addition of 0 2 ml of normal platelet suspension (380 000 platelets per µl washed* twice) The thrombin generation normal Recalcification time 4 min 40 sec

Curve E The patient s platelet rich plasma stored at −20 C for 8 days showed a some what improved thrombin generation which however did not reach maximum till after 10 minutes The thrombin formed was thereafter quickly inactivated Recalcifica tion time *4 min 26 sec*

Curve F Addition of 0 2 ml of readsorbed normal serum No improvement of the *thrombin generation* Recalcification time *6 min 45 sec*

Curve G Addition of adsorbed bovine plasma as well as serum gave no further im provement of the thrombin generation than addition of bovine plasma alone Re calcification time 3 min 25 sec

0 2 ml of this patient s plasma was able to normalise the thrombin generation *in plasma lacking the Christmas factor (fam 62) but not in plasma lacking the AHF* (fam 79 and fam 84)

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

3 Born 1921 The patient has always bruised easily At the age of 4 years he had a prolonged bleeding from a wound on his left heel A dental extraction was followed by oozing haemorrhage lasting 8 days Especially between the ages of 14 and 18 there were frequent haemathroses *particularly in the left knee and in* both elbows The left knee has a 15 degrees flexion defect At the age of 32 the patient had haematuria for *3 weeks He has never had melaena nor haematemesis The patient has previously had* frequent episodes of prolonged epistaxis The patient has not been particularly troubled by his disease since the age of 20 He is a mechanic and is able to support his *family*

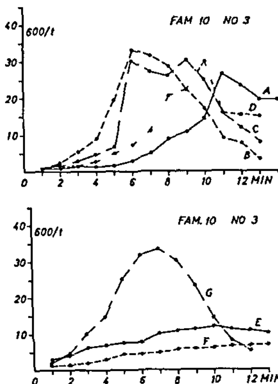
Blood analyses 1953 (The Kommunehospitalet Dept 3) Clotting time 8 min 30 sec 14 min 15 min 30 sec Thrombocytes 209 000 *Bleeding* time 2 *min* 30 sec Fibrinogen 0 36 %

Present investigations Quick s prothrombin time 24 25 24 sec (control 20 sec) *Prothrombin proconvertin (Owren) 100 % Thrombocytes 728 000 per µl of plasma Clot* ting time for whole blood at 37 C 20 min Recalcification time in dilute plasma 6 min 50 sec The thrombin generation test showed a lag period *of 6–8 min after which the* thrombin concentration rose relatively fast and reached high values but not till after 11 minutes The beginning of inactivation was rather slow (Curve A)

Curve B Addition of 0 2 ml of adsorbed bovine plasma caused a rapid rise and fall of the thrombin concentration Recalcification time 2 min 50 sec

Curve C *Addition of 0 2 ml of serum The thrombin generation* was accelerated but the lag period was prolonged about 5 min Recalcification time 4 min 46 sec

Curve D Addition of 0 4 ml *of fresh platelet suspension (91 000 platelets per µl* of suspension washed twice) Little improvement of the thrombin generation Recalci fication time 5 min



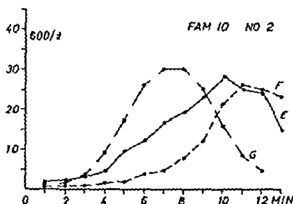
Curve E Storage of the patient s plasma with its normal platelet content at —20 C for 24 hours gave a poorer thrombin generation At the same time the recalcification time was reduced 2 min 35 sec

Addition of 0 2 ml of the patient s plasma to plasma from another patient with AHF deficiency (fam 71 no 4) did not improve the thrombin generation in the plasma of the patient concerned (curve F) Recalcification time 6 min 45 sec After 0 ml of readsorbed serum had been added to the patient s plasma the thrombin generation became perfectly normal (Curve G) The recalcification time was min 50 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

4 Born 1947 At the age of 1 year the patient was in hospital owing to prolonged bleeding from an injured upper lip He is liable to large subcutaneous extravasations of blood In 1948 the patient was admitted to hospital on account of a large sub utaneous haematoma over the right ligament extending into the right flank He has never had remarkable epistaxis There have been a few episodes of haematuria but never melaena nor haematemesis From the age of 1 year he fairly often had posttraumatic haemar throses especially in the elbows and ankles more rarely in the knees These have not yet caused permanent changes Cuts never bled extraordinarily The patient has never received a blood transfusion In addition to the bleeding tendency the patient suffers from asthma He has missed about one third of his school days on account of the haemophilia

Blood analyses Quick s prothrombin time 19 19 18 sec (control 18 sec) Thrombo cytes 359 000 per µl of plasma Recalcification time in dilute plasma 7 min The thrombin generation test showed a very slow rise of the thrombin concentration which did not reach high values (Curve A)



Curve D Addition of 0 2 ml of normal platelet suspension (380 000 platelets per μl washed twice) The thrombin generation normal Recalcification time 4 min 40 sec

Curve E The patient s platelet rich plasma stored at −20 C for 8 days showed a some what improved thrombin generation which however did not reach maximum till after 10 minutes The thrombin formed was thereafter quickly inactivated Recalcifica tion time 4 min 26 sec

Curve F Addition of 0 2 ml of readsorbed normal serum No improvement of the thrombin generation Recalcification time 6 min 45 sec

Curve G Addition of adsorbed bovine plasma as well as serum gave no further im provement of the thrombin generation than addition of bovine plasma alone Re calcification time 3 min 25 sec

0 2 ml of this patient s plasma was able to normalise the thrombin generation in plasma lacking the Christmas factor (fam 62) but not in plasma lacking the AHF (fam 79 and fam 84)

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

3 Born 1921 The patient has always bruised easily At the age of 4 years he had a prolonged bleeding from a wound on his left heel A dental extraction was followed by oozing haemorrhage lasting 8 days Especially between the ages of 14 and 18 there were frequent haemathroses particularly in the left knee and in both elbows The left knee has a 15 degrees flexion defect At the age of 32 the patient had haematuria for 3 weeks He has never had melaena nor haematemesis The patient has previously had frequent episodes of prolonged epistaxis The patient has not been particularly troubled by his disease since the age of 20 He is a mechanic and is able to support his family

Blood analyses 1953 (The Kommunehospitalet Dept 3) Clotting time 8 min 30 sec 14 min 15 min 30 sec Thrombocytes 209 000 Bleeding time 2 min 30 sec Fibrinogen 0 36 %

Present investigations Quick s prothrombin time 24 25 24 sec (control 20 sec) Prothrombin proconvertin (Owren) 100 % Thrombocytes 728 000 per μl of plasma Clot ting time for whole blood at 37 C 20 min Recalcification time in dilute plasma 6 min 50 sec The thrombin generation test showed a lag period of 6–8 min after which the thrombin concentration rose relatively fast and reached high values but not till after 11 minutes The beginning of inactivation was rather slow (Curve A)

Curve B Addition of 0 ? ml of adsorbed bovine plasma caused a rapid rise and fall of the thrombin concentration Recalcification time 2 min 50 sec

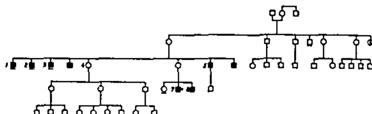
Curve C Addition of 0 2 ml of serum The thrombin generation was accelerated but the lag period was prolonged about 5 min Recalcification time 4 min 46 sec

Curve D Addition of 0 4 ml of fresh platelet suspension (91 000 platelets per μl of suspension washed twice) Little improvement of the thrombin generation Recalci fication time 5 min

The reasons why the family was not included in the group with slight AHF deficiency were partly that serum did not completely normalise the thrombin generation in two of the patients and partly that two patient (nos 2 and 4) were much more affected than the remaining patients within the group with slight AHF deficiency

Clinically this family represents an intermediate form between the group designated as AHF deficiency and that designated as slight AHF deficiency

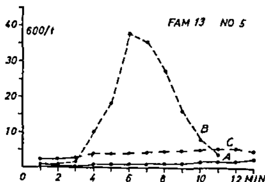
Family 13



5 Born 1900 Propositus The first abnormal bleeding was observed within the first year of life In infancy he had had copious oozing haemorrhages from the gums and nose There have been several haemarthroses chiefly in the ankles elbows and knees The patient has gradually developed permanent limitation of mobility of the ankles and the knee joints He has frequent episodes of haematuria and has once had an intra abdominal haemorrhage A muscular bleeding in the right forearm was followed by median nerve paralysis He has had melaena once There has been prolonged oozing bleeding after dental extra tion The patient is liable to ecchymoses He has received many blood transfusions The patient owns a farm which until recently was administered by himself and he also took part in the work He does not receive disablement benefit The disease has hardly grown milder in the course of years

Blood analyses 1927 (The Rigshospitalet Dept B) Clotting time ac ording to Howell Gram 18 min Thrombocytes 364 000

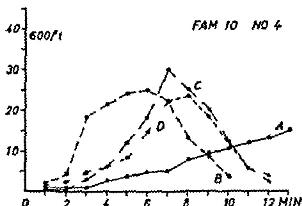
Present investigations Quick s prothrombin time 17 18 18 sec (control 18 sec) Thrombocytes 146 000 per µl of plasma Recalcification time in dilute plasma 16 min The thrombin generation test showed practically no thrombin generation in the course of 13 minutes (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal The recalcification time fell to 3 min 25 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 3 min 30 sec

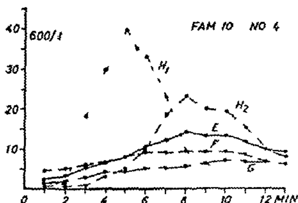
Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal *Recalcification time 2 min 20 sec*

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Re calcification *time fairly long 4 min 30 sec*

Curve D Addition of 0 2 ml of readsorbed serum The thrombin generation normal *Recalcification time 3 min 30 sec*



Curve E Addition of 0 2 ml of heated readsorbed serum No definite improvement of the thrombin generation Recalcification time 2 min 50 sec

Curve F Addition of 0 2 ml of frozen platelet suspension (272 000 platelets per µl) No improvement of the thrombin generation Recalcification time 1 min 20 sec

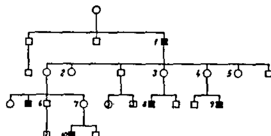
The patient s plasma was unable to normalise the thrombin generation in plasma from another patient lacking the AHF (Curve G) (fam 71 no 4) Recalcification time 5 min 45 sec On the other hand the patient s plasma normalised the thrombin generation of plasma from a patient with Christmas disease (fam 22 no 14) The latter patient s curves before and after addition of the present patient s plasma are H and H_1 in the chart

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Comments

This family ought to have been classified in the group of slight AHF deficiency partly owing to a considerable though delayed thrombin generation at least in the plasma of two of the patients and partly because the thrombin generation besides be coming normal after addition of adsorbed bovine plasma improved appreciably after addition of serum plasma from patient no 4 even attained a normal thrombin generation

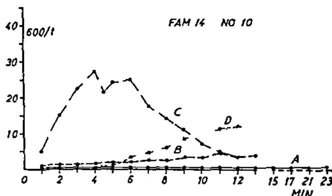
Family 14



10 Born 1938 The first haemorrhage was from the gums on dentition at the age of 6 months From his second year of life there have been frequent haemarthroses which have left contractures of the elbows and knees X ray of the knee joints at the age of 17 revealed narrowed joint cavities and irregular articular surfaces Haematuria and melaena have been observed several times The patient is liable to subcutaneous haema tomas 17 years old he experienced an intra abdominal haemorrhage after a fall This was followed by paresis and weakness of the left leg Further there was loss of sensibility on the anterior and medial sides of the leg These symptoms have subsided again The patient has received a single transfusion He is reading for the general certificate

Blood analyses 1955 (The Frederiksberg Hospital Med Dept B) Thrombocytes 637 000 Clotting time 4 min

Present investigations Quick s prothrombin time 21 21 19 sec (control 18 sec) Prothrombin proconvertin (Owren) 100 % Recalcification time in dilute plasma 22 min The thrombin generation test showed negligeable thrombin generation the first 23 minutes (Curve A)



Curve B Addition of 0 2 ml of readsorbed bovine plasma The thrombin generation poor Recalcification time 7 min

Curve C Addition of 0 2 ml of serum from a patient with AHF deficiency (fam 2 no 11) The thrombin generation normal Recalcification time 1 min

Curve D Addition of 0 2 ml of heated readsorbed serum The thrombin generation poor Recalcification time 6 min 30 sec

Diagnosis Christmas factor deficiency (Christmas disease)

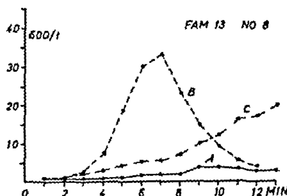
8 Born 1930 The patient has had frequent haemarthroses since he began to crawl especially in the knees and ankles The left knee is slightly deformed with extension defect of a few degrees About the age of 18 months he had prolonged oozing gingival haemorrhages Periodically he has been liable to bruise He has had three episodes of haematuria but never melaena nor haematemesis The patient has never had a tooth extracted Catarrhal diseases have often been attended by prolonged epistaxis In 1940 the patient fell and hurt his back This was followed by pain in the lumbar region After 8 days there was involuntary discharge of urine and faeces and a few days later the patient developed deep areflexia of both legs and hypaesthesia of the abdomen extending as far as the fourth lumbar segment Neurologic diagnosis Haematomyelia (The Kommunehospitalet Copenhagen Dept 7) The neurological changes subsided in the course of 2–3 months but contracture persists of the right ankle which is bent and inverted The patient has graduated in law and is able to support himself

Blood analyses 1931 (The Rigshospitalet Paediatric Departm) Clotting time according to Howell Gram 23 min Bleeding time 5½ min Thrombocytes 289 000

Andreassen 1941 Clotting time 10½ min – 27 min Thrombocytes 381 000 1947 Clotting time 8–17 min Clot loose

1954 (The Rigshospitalet Dept A) Fibrinogen 0 59 % Bleeding time 2½ min Prothrombin 92–105 % Prothrombin in serum 142 %

Present investigations Quick s prothrombin time 18 18 19 sec (control 18 sec) Thrombocytes 243 000 per μl of plasma Recalcification time in dilute plasma 15 min The thrombin generation test showed practically no thrombin generation in the course of 13 minutes (Curve A)

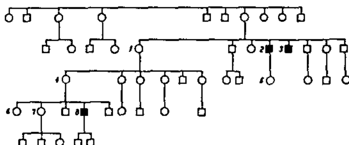


Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 38 sec

Curve C Addition of 0 2 ml of normal serum Very slow rise of the thrombin concentration after a lag period of 6 minutes Recalcification time 6 min 14 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

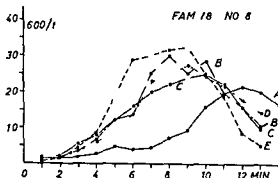
Family 18



8 Born 1939 Propositus The patient s first abnormal bleeding occurred at the age of 2 years as a posttraumatic haematoma in the skin He has had numerous haemorrhages since many of which have been life threatening and necessitated admission to hospital In infancy he most often had mucosal bleedings from the nose and mouth Later haemar throses predominated chiefly in the finger joints ankles and knees resulting in the latter joints in permanent limitation of mobility At the age of 8 he bled for 10 days after dental extraction The patient has had several copious intramuscular bleedings requiring prolonged confinement to bed Haematuria has been observed but no other intra abdominal haemorrhage The patient has been given several blood transfusions Although the patient has been and still is greatly troubled by his disease he manages well as a master artisan He has three workshops and a large staff

Blood analyses 1949 (The Rigshospitalet Dept A) Clotting time 8 min Bleeding time 5½ min Capillary resistance 8 petechiae Prothrombin time 22 sec (control 22 sec)

Present investigations Quick s prothrombin time 21 20 20 sec (control 20 sec) Clotting time for whole blood at 37 C 45 min Recalcification time in dilute plasma 7 min The thrombin generation test showed some thrombin generation but only after a lag period of 7 min (Curve A)



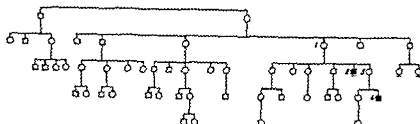
Curve B Addition of 0 ml of adsorbed bovine plasma The thrombin generation nor mal Recalcification time 3 min 15 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation considerably improved but maximum concentration was not attained till after 10 minutes Recalci fication time 3 min 25 sec

Curve D Addition of 0 2 ml of readsorbed serum The thrombin generation nearly normal Recalcification time 4 min

Curve E Addition of 0 2 ml of heated readsorbed serum The thrombin generation normal the lag period perhaps slightly prolonged Recalcification time 4 min 10 sec

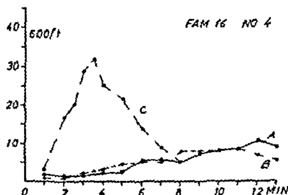
Family 16



4 Born 1936 A liability to large subcutaneous haematomas was noticed already at the age of 1 month From the age of 1 year he had prolonged oozing haemorrhages from the gums and nose and since the age of 4 years frequent bleedings in the knees elbows and ankles Within the last few years there have also been frequent bleedings into the finger joints At the age of 13 the patient s left knee joint was submitted to arthrodesis since which there have been no haemorrhages here He has repeatedly had haematuria but never haematemesis nor melaena He has had one episode of bleeding sublingually and in the neck with no attending respiratory trouble The patient is given a transfusion every 5 weeks or so and feels that it helps him He is apprenticed to a watch maker and is pleased with his work

Blood analyses 1940 (Holstebro Hospital) Clotting time according to Howell Gram 18 min 1951 Thrombocytes 205 000

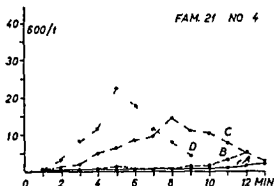
Present investigations Quick s prothrombin time 23 23 23 sec (control 19 sec) *Clotting time for whole blood at 37 C 22 min Recalcification time in dilute plasma* 8 min 30 sec The thrombin generation test showed very little thrombin generation (Curve A)



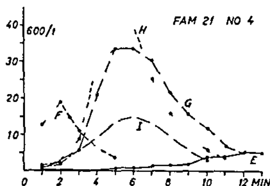
Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 5 min

Curve C *Addition of 0 2 ml of adsorbed bovine plasma* The thrombin generation normal Recalcification time 1 min 49 sec

Diagnosis *Deficiency of the antihaemophilic factor (classical haemophilia)*



Curve B Addition of 0 ? ml of normal serum The thrombin generation poor Recalcification time 7 min
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation improved though the concentration rose slowly Recalcification time 2 min 24 sec
Curve D Simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 2 ml of heated readsorbed serum The thrombin generation normal Recalcification time 1 min 36 sec

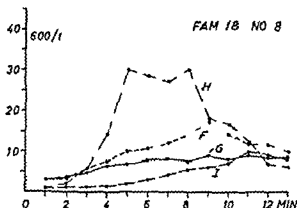


Curve E Addition of 0 2 ml of heated readsorbed serum alone gave no definite improvement of the thrombin generation The recalcification time fell however to 5 min 45 sec
Curve F Addition of 0 2 ml of adsorbed bovine plasma to the patients plasma with its normal content of platelets after storage at −20 C for 7 days The thrombin generation was greatly accelerated Recalcification time 1 min

Signs of an inhibitor were not demonstrable The patients plasma did not delay the thrombin generation in a control plasma Curves G and H show the thrombin generation in the control plasma before and after addition of the patients plasma

Curve I shows the thrombin generation in the patients plasma after addition of 0 2 ml of adsorbed bovine plasma and addition of the platelets of the patients own plasma after these had been washed four times There seemed to be some acceleration of the thrombin generation compared with curve C The recalcification time was unchanged 2 min 25 sec

Diagnosis Deficiency of the ant haemophilic factor plus freezing serum factor



Curve F Addition of the patient's own washed platelets to the plasma from which they had been isolated The thrombin generation somewhat accelerated but rather poor Recalcification time normal 3 min 50 sec

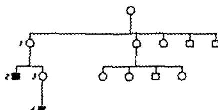
Curve G The thrombin generation poor in the patient's platelet rich plasma after this had been stored at −20 C for 24 hours Recalcification time 2 min 30 sec

Curve H 0 2 ml of plasma from a patient with Christmas disease normalised the thrombin generation Recalcification time 3 min 30 sec

Curve I 0 5 ml of plasma from a patient with AHF deficiency did not normalise the thrombin generation Recalcification time 6 min 50 sec

Diagnosis Slight antihaemophilic factor deficiency

Family 21



4 *Born 1941 A bleeding tendency manifested itself from the age of 5 months by* large apparently spontaneous haematomas on the body In 1941 the patient was in *hospital* with a large *haematoma in the left axilla One year old he had prolonged* haemorrhage after paracentesis The patient has had frequent episodes of gingival hae *morrhage melaena haematuria epistaxis and haemarthroses He has had prolonged* bleed ings after cuts There is extension defect of both elbows and pronounced flexion defect *of the left knee with halisteresis and arthrosis of the bones*

Blood analyses 1941 (The Alborg Amtssygehus) Clotting time direct 70 min Thrombocytes 500 000–382 000 *Bleeding time 3 min* Capillary resistance normal 1942 Clotting time 2 hours Prothrombin time 80

1957 (The Rigshospitalet Dept A) Thrombocytes 224 000 Clotting time 15 min Bleeding time 6 min Capillary resistance No petechiae Fibrinogen 0 13 %

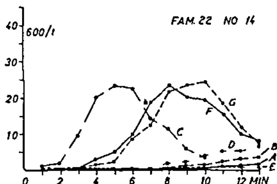
Present investigations Quick's prothrombin time 16 16 16 sec (control 16 sec) Thrombocytes 486 000 per µl of plasma Recalcification time in dilute plasma 13 min 10 sec *The thrombin* generation test showed no thrombin generation (Curve A)

Family 22

14 Born 1916 One year old the patient had his first abnormal bleeding In infancy he had frequent oozing gingival haemorrhages and repeatedly spontaneous subcutaneous haematomas on the body From the age of 2–3 years there were increasingly frequent painful haemorrhages into the knees and ankles Flexion contracture has developed in the right knee and elbow He has had several episodes of prolonged haematuria but never haematemesis nor melaena

The disease is stated to have grown somewhat milder in the course of years The patient has had only 14 days lost through sickness a year within the past 6 eyars He is a tailor and can support his family He has been given only one transfusion

Blood analyses Quick s prothrombin time 17 18 18 sec (control 18 sec) Thrombocytes 272 000 per µl of plasma Recalcification time in dilute plasma 10 min 45 sec The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma No improvement of the thrombin generation Recalcification time 9 min 4? sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 2 min 30 sec

Curve D Addition of 0 2 ml of frozen platelet suspension The thrombin generation poor Recalcification time 8 min 30 sec

Curve E Freezing of the plasma with its normal content of platelets down to −20 C gave no improvement of the thrombin generation

Curve F The thrombin generation in plasma containing 167 000 thrombocytes per µl was somewhat improved after freezing The lag period was about 5 minutes however Recalcification time 4 min 30 sec

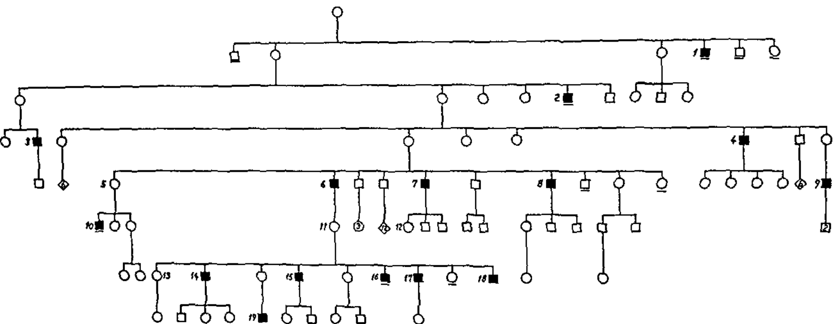
Curve G Addition of 0 2 ml of heated readsorbed normal serum to this plasma gave no further improvement of the thrombin generation Recalcification time 5 min ?5 sec

Diagnosis Christmas factor deficiency (Christmas disease)

15 Born 19?5 18 months old the patient had prolonged oozing haemorrhage from a wound in the upper lip Since there have been numerous bleedings from the nose and to a less extent from the gums From school age there were increasingly frequent and painful haemarthroses most often in the knee joints which have gradually become permanently deformed with extension defect There have also been haemorrhages in the ankles and elbows The patient has never had haematemesis melaena nor haematuria In 1955 there was spontaneous haemothorax The disease is alleged to have grown milder with increasing years The patient receives disablement benefit however

Blood analyses 1956 Quick s prothrombin time 16 17 18 sec (control 18 sec) Thrombocytes 475 000 per µl of plasma Recalcification time in dilute plasma 11 min 56 sec The thrombin generation test showed negligeable thrombin generation (Curve A)

Family 22



Curve C Addition of 0 ? ml of normal serum The thrombin generation normal Recal cification time 3 min

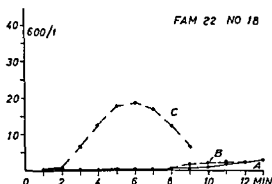
Curve D Addition of 0 2 ml of heated readsorbed serum The thrombin generation considerably improved but the lag period was 4 min Recal ification time 4 min ?0 sec

Freezing experiments gave the same results as in the cases of the previously described patients of this family

Diagnosis Christmas factor deficiency (Christmas disease)

18 Born 1940 3 months old the patient had an apparently spontaneously developed haematoma on the breast and 6 months old a likewise apparently spontaneous haematoma in the scrotum extending to the anterior surface of the abdomen

Blood analyses Quick s prothrombin time 19 18 19 sec (control 18 sec) Thrombo cytes 317 000 per μl of plasma Recalcification time in dilute plasma 11 min 30 sec The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor *Recalcification time 9 min 25 sec*

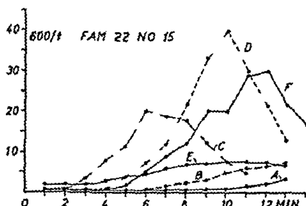
Curve C Addition of 0 ? ml of normal serum The thrombin generation normal Recal cification time 3 min 15 sec

As was the case for the remaining patients of this family freezing of the platelet rich plasma gave no essential improvement of the thrombin generation

Diagnosis Christmas factor deficiency (Christmas disease)

19 Born 1941 A bleeding tendency was noticed from early infancy manifesting it self by subcutaneous haematomas and prolonged haemorrhage following dental extraction The patient has had several episodes of epistaxis of up to 3 weeks duration He has had one episode of haematuria but never haematemesis nor melaena There have been numerous haemorrhages into the knees elbows shoulders and ankles A permanent flexion contracture has occurred of the right knee joint which displays lateral subluxation There is extension defect of the left elbow The forearm is fixed in slight pronation There is limited mobility of both ankles The patient is greatly troubled by his disease He is apprenticed to a watch maker

Blood analyses Quick s prothrombin time 20 19 19 sec (control 18 sec) Thrombo cytes 338 000 per μl of plasma Recalcification time in dilute plasma 10 min 45 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 7 min 20 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 3 min 20 sec

Curve D Addition of 0 2 ml of heated normal serum caused essential improvement of the thrombin generation but the lag period was rather long 4–5 min Recalcification time 5 min

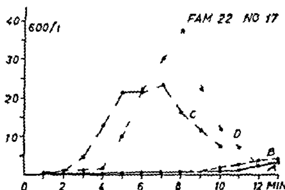
Curve E Freezing of the plasma with its normal content of platelets did not improve the thrombin generation Recalcification time 5 min 15 sec

*Curve F Freezing of platelet poor plasma (21 000 platelets per μl) The thrombin genera*tion improved but the lag period was prolonged Recalcification time 6 min 20 sec

Diagnosis Christmas factor deficiency (Christmas disease)

17 Born 1933 2 years old the patient had a large apparently spontaneously developed haematoma on the forehead He has had numerous gingival haemorrhages From the age of 5–6 years there were increasingly frequent haemarthroses chiefly in the knees *feet and elbows There is limited mobility of both elbow joints but unlimited of the* other joints He has experienced numerous spontaneous haemorrhages in the muscles and in the skin The patient has never had haematuria haematemesis nor melaena He receives disablement benefit but is partially fit to work as a tailor

Blood analyses Quick s prothrombin time 18 18 19 sec (control 18 sec) Thrombocytes *277 000 per μl of plasma Recalcification time in dilute plasma* 11 min 30 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 9 min

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recal cification time 3 min

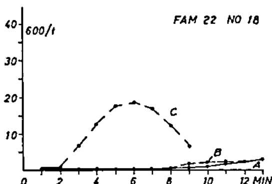
Curve D Addition of 0 2 ml of heated readsorbed serum The thrombin generation considerably improved but the lag period was 4 min Recalcification time 4 min 20 sec

Freezing experiments gave the same results as in the cases of the previously described patients of this family

Diagnosis Christmas factor deficiency (Christmas disease)

18 Born 1940 3 months old the patient had an apparently spontaneously developed haematoma on the breast and 6 months old a likewise apparently spontaneous haematoma in the scrotum extending to the anterior surface of the abdomen

Blood analyses Quick s prothrombin time 19 18 19 sec (control 18 sec) Thrombo cytes 317 000 per µl of plasma Recalcification time in dilute plasma 11 min 30 sec The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 9 min 25 sec

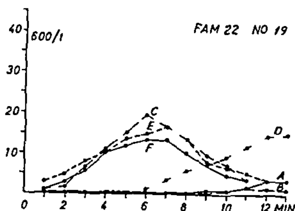
Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recal cification time 3 min 15 sec

As was the case for the remaining patients of this family freezing of the platelet rich plasma gave no essential improvement of the thrombin generation

Diagnosis Christmas factor deficiency (Christmas disease)

19 Born 1941 A bleeding tendency was noticed from early infancy manifesting it self by subcutaneous haematomas and prolonged haemorrhage following dental extraction *The patient has had several episodes of epistaxis of up to 3 weeks duration He has* had one episode of haematuria but never haematemesis nor melaena There have been numerous haemorrhages into the knees elbows shoulders and ankles A permanent flexion contracture has occurred of the right knee joint which displays lateral subluxation There is extension defect of the left elbow The forearm is fixed in slight pronation There is limited mobility of both ankles The patient is greatly troubled by his disease He is apprenticed to a watch maker

Blood analyses Quick s prothrombin time 20 19 19 sec (control 18 sec) Thrombo cytes 338 000 per µl of plasma Recalcification time in dilute plasma 10 min 45 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor *Recalcification time 11 min 30 sec*

Curve C *Addition of 0 2 ml of normal serum The thrombin generation normal* Recalcification time 3 min

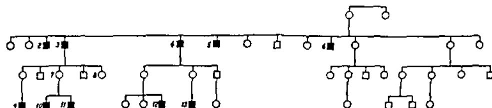
Curve D Addition of 0 2 ml of heated readsorbed serum The thrombin generation poor Recalcification time 8 min 30 sec

Curve E Addition of 0 2 ml of frozen platelet suspension containing 178 000 platelets per μl of suspension The thrombin generation normal Recalcification time 1 min 30 sec

Curve F Addition of 0 2 ml of the patient s own serum (stored for 1 year) The thrombin generation normal Recalcification time 2 min

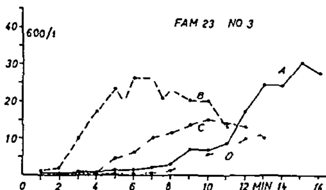
Diagnosis Christmas factor deficiency (Christmas disease)

Family 23



3 Born 1895 The patient s bleeding tendency first manifested itself at the age of 13 or 14 as a profuse haemarthrosis This was followed later by numerous haemarthroses chiefly in the knees and ankles as well as in the elbows on heavy work There is limited mobility of the right elbow joint which is 35 degrees short of full extension *The right knee joint can be moved from 180 to 110 degrees The patient has had several* prolonged gingival haemorrhages as well as several severe intramuscular bleedings An intramuscular bleeding in the right thigh in 1934 became infected and had to be incised The operation was followed by prolonged bleeding which did not cease till after repeated blood transfusions The patient has several varices on the left lower leg These have ruptured spontaneously two or three times but the bleeding each time stopped soon after ordinary bandaging The patient s disease has grown milder in the course of years He is able to provide for himself and his family as a furnace man

Blood analyses Quick s prothrombin time 19 19 19 sec (control 20 sec) Prothrombin proconvertin (Owren) 90 % Recalcification time in dilute plasma 7 min 30 sec The thrombin generation test showed after a lag period of 8 min an accelerated thrombin generation A fairly high concentration was reached (Curve A)

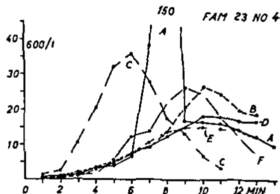


Curve B Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 3 min
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation somewhat accelerated without becoming normal Recalcification time 4 min 45 sec
Curve D Addition of 0 ⌞ ml of heated readsorbed serum The thrombin generation poor Recalcification time 10 min

Diagnosis Christmas factor deficiency (Christmas disease)

4 Born 1896 The first abnormal bleeding was observed when the patient at the age of 16–17 had prolonged haemorrhage following dental extraction He has had frequent haemorrhages since in the forms of subcutaneous haematomas epistaxis haemarthroses chiefly in the knees and elbows which have limited mobility and are deformed The right knee is completely stiff There have been occasional episodes of haematuria lasting a few weeks but never haematemesis nor melaena He has had several intramuscular bleedings most often in the throat, wh re they have now and then caused respiratory troubles In 1953 there was spontaneous haemorrhage from a laryngeal papilloma The bleeding stopped in response to a coagulant He was not operated on He had been a soldier for 10¹₂ months when he was rejected because of haemorrhage in the right knee joint. The disease has grown milder since his 36th year The patient is a grocer and able to support himself and a family

Blood analyses Quick s *prothrombin time* 19 20 21 *sec* (*control* 17 *sec*) *Thrombocytes* 401 000 per μl of plasma Recalcification time in dilute plasma 4 min The thrombin generation test showed a very steep rise of the thrombin concentration after a lag period of 6 min The concentration fell rather soon again (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation delayed Recalcification time 5 min 10 sec

Curve C Addition of 0 2 ml of serum The thrombin generation normal Recalcification time 2 min 46 sec

Curve D On adding heated readsorbed serum the thrombin generation was rather poor Recalcification time 4 min 26 sec

Curve E Addition of 0 2 ml of platelet suspension (272 000 per µl of suspension) The thrombin generation poor Recalcification time 3 min 46 sec

Curve F The thrombin generation in plasma stored at −20 C with its normal content of platelets was almost normal though somewhat delayed Recalcification time 5 min 10 sec

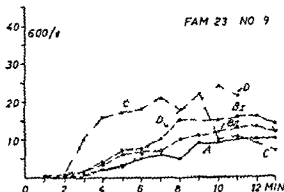
Diagnosis Christmas factor deficiency (Christmas disease)

9 Born *1935* Propositus The patient had his first abnormal bleeding at the age of 5 months when a large haematoma occurred on one leg He has had numerous haemorrhages since especially from the gums and nose which often necessitated hospitalisation Since the age of 2 years he has repeatedly been in hospital on account of bleedings into the knees wrists elbows ankles and hip joints The bleedings arose partly spontaneously and partly following injuries Permanent changes with limited mobility have gradually developed in both knee joints and both ankles The patient has had a single episode of melaena and haematemesis and several times haematuria He is able to provide for himself as an office clerk

Blood analyses 1942 Clotting time 15–22½ min Clot loose Blood platelets 400 000 Bleeding time 11 min

1949 Thrombocytes 400 000 Serum calcium 9 3 mg % Gøthlin 95 mm mercury for 3 min no petechiae

Present investigations Quick s prothrombin time 17 18 19 sec (control 18 sec) Thrombocytes 290 000 per µl of plasma Recalcification time in dilute plasma 4 min 30 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B I Addition of 0 2 ml of plasma from pt no 3 fam 4 (Christmas disease) The thrombin generation poor Recalcification time 4 min

Curve B II Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 4 min 30 sec

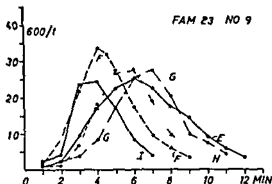
Curve C Addition of 0 2 ml of plasma from a patient with AHF deficiency (fam 69) The thrombin generation normal Recalcification time 3 min

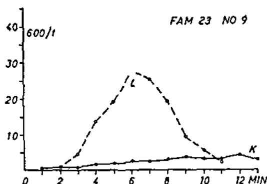
On examination 2 years later the course of the thrombin generation corresponded to curve D where a considerable amount of thrombin is seen to have been generated after a prolonged lag period (about 5 minutes) Recalcification time 5 min 30 sec

After addition of either adsorbed bovine plasma or serum or heated readsorbed serum the thrombin generation became normal (Curves E F and G respectively) The recalcification times were 2 min 30 sec 2 min 15 sec and 3 min 30 sec respectively

Curve H Addition of the patient s own washed platelets to the plasma from which they originated likewise normalised the thrombin generation Recalcification time 3 min

Curve I In plasma stored at –20 C with its normal platelet content the thrombin generation was normal *Recalcification time 2 min 15 sec*





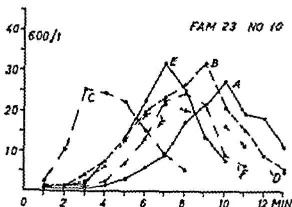
Curve K 0 2 ml of the patient s plasma was unable to normalise the thrombin generation in plasma from another Christmas patient (fam 85 no 1)

Curve L Addition of 1 0 ml of the patient s plasma normalised the thrombin generation in plasma lacking the Christmas factor

Diagnosis Slight Christmas factor deficiency (Christmas disease)

10 Born 1946 The patient has been liable to bruises since his first year of life He has had one episode of gingival bleeding which stopped spontaneously There has been no abnormal epistaxis and never haemarthroses He once had a large haematoma on the right thigh following an injury There has been no haemorrhage from the gastro intestinal or the urinary tract

Blood analyses Quick s prothrombin time 22 22 22 sec (control 19 sec) Thrombocytes 804 000 per µl of plasma Recalcification time in dilute plasma 4 min 45 sec The thrombin generation test showed a rather steep rise and fall of the thrombin concentra tion after a lag period of 5-6 min (Curve A)



Curve B *Addition of 0 2 ml of adsorbed bovine plasma shortened the lag period but* maximum thrombin concentration was not attained till after 9 min Recalcification time 3 min 35 sec

Curve C *Addition of 0 2 ml of normal serum The thrombin generation normal* Recalcification time 1 min 50 sec

Curve D Addition of 0 2 ml of readsorbed heated serum The thrombin generation normal Recalcification time 3 min 15 sec

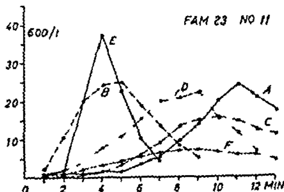
Curve E Addition of 0 2 ml of frozen platelets from a patient with Christmas disease (fam 4 no 10) The thrombin generation normal Recalcification time 3 min 55 sec

Curve F The thrombin generation was practically normal in the patient s plasma with its normal content of platelets after storage at −20 C for 20 hours Recalcification time 4 min 30 sec

The patient s plasma could not normalise the thrombin generation in plasma from another Christmas patient (fam 85 no 1)

Diagnosis Slight Christmas factor deficiency (Christmas disease)

11 Born 1950 At the age of 7 months the patient bled from the frenulum labii superior following an injury He has repeatedly experienced extravasation of blood into the ankles after spraining once extravasation into the left metacarpophalangeal and basic joints He has never had haematuria melaena nor epistaxis 6 years old the patient was admitted to hospital on account of haemorrhage after having bit his tongue Haemostasis was obtained after local treatment with stypven



Blood analyses (The Københavns Amtssygehus Hellerup Paediatric Dept) Thrombocytes 370 000 Prothrombin index 67 ,

Present investigations Quick s prothrombin time 24 23 23 sec (control 20 sec) Thrombocytes 503 000 per µl of plasma Recalcification time in dilute plasma 6 min 50 sec The thrombin generation test showed a rather steep rise of the thrombin concentration after a lag period of 5–6 min (Curve A)

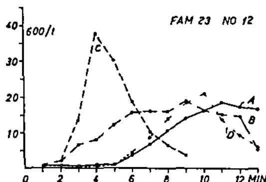
Curve B Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 2 min
Curve C Addition of heated readsorbed serum The thrombin generation slow Recalcification time 5 min 40 sec
Curve D Addition of 0 2 ml of ad orbed bovine plasma The thrombin generation normal Recalcification time 3 min
Curve E After the plasma had been stored with its normal content of platelets at –20 C for 70 hours the thrombin generation was normal Recalcification time 2 min 30 sec
Curve F 0 2 ml of the patient s plasma was unable to normalise the thrombin generation in plasma from a patient with Christmas disease (fam 85)

Diagnosis Slight Christmas factor deficiency (Christmas disease)

12 Born 1950 Since the age of 1–2 months the patient has had prolonged bleedings even from minor cuts as well as a tendency to large bruises He has had numerous large circumscribed swellings in the scalp of posttraumatic as well as apparently spontaneous origin There have been several bleedings into the elbow joints resulting in transitorily limited mobility but no haemarthroses elsewhere The patient has never bled from the nose gums gastro intestinal tract or urinary system He has been admitted to hospital several times on account of large haematomas of the head and neck

Blood analyses (The Centralsygehuset Slagelse Surg Dept) Thrombocytes 318 000

Present investigations Recalcification time in dilute plasma 6 min 45 sec Quick s prothrombin time 23 23 23 sec (control 20 sec) The thrombin generation test showed a slow rise of the thrombin concentration after a lag period of 5 minutes (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation essentially improved Recalcification time 3 min 25 sec
Curve C Addition of 0 2 ml of serum The thrombin generation normal Recalcification time 3 min 10 sec
Curve D Addition of heated readsorbed serum The thrombin generation delayed Recalcification time 6 min 30 sec

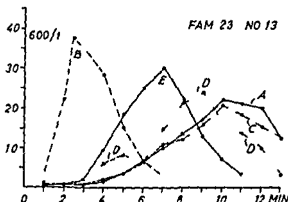
The patient s plasma normalised the thrombin generation in plasma with AHF deficiency (fam 75 no 1 fam 76 no 1) but not that in plasma lacking the Christmas factor (fam 85)

Diagnosis Slight Christmas factor deficiency (Christmas disease)

13 Born 1953 At the age of 2½ years the patient bled for 14 days from the frenulum labii superior He has been liable to ecchymoses since 3 years old he was admitted to hospital owing to haemorrhage from a large subcutaneous occipital haematoma The patient has had no mucosal haemorrhages haemarthroses haematuria nor melaena

Blood analyses (The Centralsygehuset Slagelse Med Dept) 1956 Clotting time 3 min Thrombocytes 371 0000 Prothrombin proconvertin (Owren) 72 %

Present investigations Quick s prothrombin time 18 18 17 sec (control 17 sec) Thrombocytes 192 000 per µl of plasma Recalcification time in dilute plasma 5 min 15 sec The thrombin generation test showed a slow rise of the thrombin concentration after a lag period of 4 min (Curve A)



Curve B Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 1 min 30 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation delayed Recalcification time 4 min 35 sec

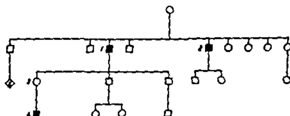
Curve D Addition of 0 2 ml of heated readsorbed serum The thrombin generation delayed the lag period about 5 min Recalcification time 4 min 15 sec

Curve E Storage of the plasma with its normal content of platelets at −20 C for 15 months resulted in a perfectly normal thrombin generation Recalcification time 3 min 40 sec

The patient s plasma did not normalise the thrombin generation in plasma from a Christmas patient (fam 4 no 10) but that in plasma from a Hageman patient (fam 86 no 1)

Diagnosis Christmas factor deficiency (Christmas disease)

Family 24

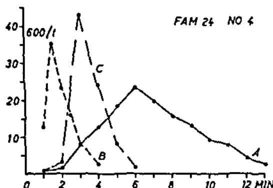


The family is of foreign origin and settled in this country in 19 0 After the Second World War several members of the family emigrated again

4 Born 1938 Propositus From the age of 2 months the patient had frequent episodes of epistaxis through some periods daily Now and then he has had large subcutaneous haematomas on the body but never haemarthroses melaena haematemesis haematuria nor intramuscular bleedings The patient cannot tell now when he had his last episode of bleeding He serves a commercial apprenticeship and manages well

Blood analyses 194? (*Andreassen*) Clotting time 10–16½ min Bleeding time 4 min Thrombocytes 310 000

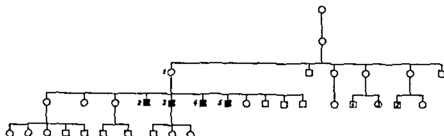
Present investigations Quick's prothrombin time 18 18 18 sec (control 18 sec) Thrombocytes 272 000 per µl of plasma



The thrombin generation test showed rapid formation and inactivation of thrombin (Curve A) Recalcification time 3 min 5 sec After storage at – 0 C for 72 hours the thrombin generation was accelerated in plasma having its normal content of platelets (Curve B) as well as in plasma having 3000 platelets per µl (Curve C)

Diagnosis No detectable clotting defect

Family 25



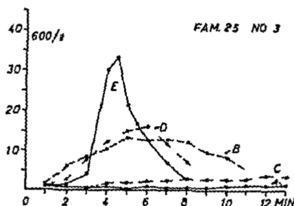
The family can be traced back to before 1850 No haemophilic members are known beyond those mentioned below

2 Born 1928 Propositus A bleeding tendency first manifested itself when the patient was 3 years old as prolonged oozing haemorrhages from the gums and nose From school age he had increasingly frequent haemarthroses in the large joints resulting especially in the ankles in limited mobility He has had one episode of haematuria accompanied by lumbar pain There have been several intramuscular haemorrhages The patient died at the age of 27 from internal bleeding after having been gored by a heifer

3 Born 1933 A bleeding tendency has been observed since the patient was 3 years old manifesting itself in the beginning as epistaxis and bleedings into the skin These often necessitated hospitalisation He has had numerous haemorrhages in the knees ankles and elbows The left knee joint is now almost immobile and the mobility of the right is greatly limited There is also limited mobility of the left elbow The patient has had haematuria but not haematemesis nor melaena He has often had gingival haemorrhages. The patient who is rather troubled by his disease receives disablement benefit He has been given many blood transfusions In 1954 the patient was submitted to appendicectomy which was followed by prolonged oozing haemorrhage

Blood analyses 1956 (The Brønderslev Hospital) Clotting time over 2 hours

Present investigations Quick s prothrombin time 15 15 15 sec (control 17 sec) Thrombocytes 169 000 Recalcification time in dilute plasma 16 min The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation improved The concentration rose and fell rather quickly without reaching high values however *Recalcification time 1 min 40 sec Addition of 0 4 ml of adsorbed bovine* plasma gave no further improvement of the thrombin generation

Curve C Addition of normal serum The thrombin generation poor Recalcification time 8 min

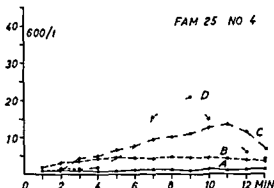
Curve D Addition of 0 2 ml of adsorbed bovine plasma to plasma with its normal *content of platelets after storage at −20 C for 10 months The thrombin generation* normal Recalcification time 2 min 50 sec

Curve E Shows the thrombin generation under the same conditions but in platelet poor plasma (7000 per µl of plasma) The thrombin generation normal

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia) and deficiency of the freezing serum factor (Christmas factor)

4 Born 1939 The patient has always bruised easily Since the age of 2 years there have been frequent episodes of epistaxis and prolonged oozing haemorrhages from cuts There was bleeding from the gums on secondary dentition The patient has had haematuria attended by lumbar pain but never haematemesis nor melaena He has had occasional bleedings into the knees and elbows which have resulted in somewhat limited mobility The patient states *that he is only moderately troubled by his disease* He does not receive disablement benefit but assists his father in his work as a herdsman

Blood analyses Quick s prothrombin time 15 15 15 sec (control 17 sec) Thrombocytes 286 000 per µl of plasma Recalcification time in dilute plasma *15 min* The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of serum The thrombin generation poor but the recalcification time fell to 3 min

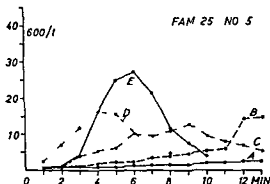
Curve C Addition of 0 2 ml of adsorbed bovine plasma caused a slow rise of the thrombin concentration Recalcification time 3 min

This experiment was repeated 14 months later on plasma stored at —?0 C with a content of 11 000 platelets per µl The thrombin generation was somewhat improved (Curve D) Recalcification time 4 min ?5 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia) plus deficiency of the freezing/serum factor (Christmas factor)

5 Born 1941 The patient s first prolonged bleeding occurred at the age of 7 years after he had injured his finger Since he has often had subcutaneous haemorrhages and long episodes of epistaxis There was prolonged bleeding on secondary dentition He has never had haematuria nor gastro intestinal haemorrhage There have been bleedings into the elbows and ankles but the mobility of the joints is not limited

Blood analyses Quick s prothrombin time 16 16 16 sec (control 17 sec) Thrombocytes 179 000 per µl of plasma Recalcification time in dilute plasma 8 min 45 sec The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of serum The thrombin concentration rose slowly Recalcification time 5 min 30 sec

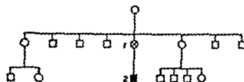
Curve C Addition of 0 2 ml of adsorbed bovine plasma A slow rise and fall of the thrombin concentration Recalcification time 3 min

Curve D Simultaneous addition of 0 _ ml of adsorbed bovine plasma and 0 2 ml of serum The thrombin generation normal Recalcification time 1 min 20 sec

Curve E Addition of 0 2 ml of adsorbed bovine plasma to plasma (26 000 thrombocytes per µl) stored at −20 C for 14 months The thrombin generation normal Recalcification time 3 min 45 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia) plus deficiency of the freezing/serum factor (Christmas factor)

Family 29

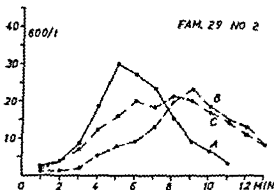


As far back as the present generation remembers there have been no cases of haemophilia in the family

2 Born 1907 Propositus A bleeding tendency was detected when the patient had a tooth extracted at the age of 10 This was followed by prolonged oozing haemorrhage for several days Similar prolonged haemorrhages followed subsequent dental extractions *There have been bleedings into the elbow joints twice after injuries but none of these have* left permanent changes There has been one episode of haematuria without attending *lumbar pain The patient was confined to bed for 3 weeks He has had a few intra* muscular bleedings The patient has been a soldier and stood the service well There have been no bleeding episodes the past 20 years No blood transfusion has ever been given The patient is a messenger (using a bicycle) and supports a family

Blood analyses 1941 (*Andreassen*) Clotting time (Burker) 15 – > 25 min Clot loose Thrombocytes 350 000

Present investigations Quick s prothrombin time 18 19 20 sec (control 19 sec) Thrombocytes 322 000 per µl of plasma Recalcification time in dilute plasma 2 min 45 sec The thrombin generation test (Curve A) showed a perfectly normal thrombin generation with a steep rise and fall of the thrombin concentration



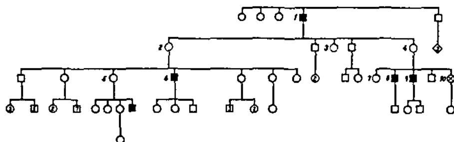
With a view to the presence of a qualitative platelet defect the thrombin generation in a control plasma was tested partly with its own platelets and partly after addition of a platelet suspension from the present patient s plasma

Curve B shows the thrombin generation curve for the control plasma

Curve C shows the thrombin generation curve for the same plasma after centrifugation for 10 min (2000 r p m) and addition of 0 4 ml of platelet suspension from the patient s plasma There seems to be no reduction of the thrombin generation

Diagnosis No detectable clotting defect

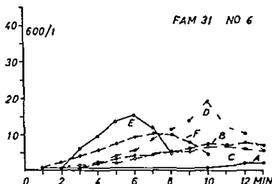
Family 31



The living members of the family are thoroughly familiar with the earlier generations There is believed to have been no cases of haemophilia beyond those set out in the pedigree

6 Born 1907 Propositus A bleeding tendency has been observed since the age of 2 years In infancy the patient had several grave gingival haemorrhages Such continued to recur throughout his growing years having abated only at the age of 25 He has had numerous haemarthroses in nearly all the large joints chiefly the knees elbows and ankles There is limited mobility of the left knee and the left elbow There has been one episode of haematuria and several intra abdominal bleedings Within recent years there have been frequent intramuscular haemorrhages without neurologic complications The patient who is a master tailor and chairman of a parish council now manages well compared with formerly He thinks that the disease has grown milder the past 10 years He has not been in hospital for many years

Blood analyses Quick s prothrombin time 18 17 17 sec (control 19 sec) Thrombocytes 17 000 (seventeen thousand) per µl of plasma Recalcification time in dilute plasma 10 min 50 sec The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma Slight improvement of the thrombin generation and a fall of the recalcification time to 4 min

Curve C Addition of normal serum Slight improvement of the thrombin generation Recalcification time 4 min 30 sec

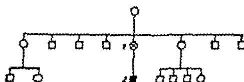
Curve D Addition of 0 4 ml of platelet suspension (170 000 platelets per µl) gave essential improvement of the thrombin generation but maximum concentration was attained fairly late Recalcification time 5 min

Curve E Simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 4 ml of platelet suspension (170 000 platelets per µl) The thrombin generation normal Recalcification time 2 min 40 sec

Curve E Addition of 0 2 ml of adsorbed bovine plasma to plasma (26 000 thrombocytes per µl) stored at −20 C for 14 months The thrombin generation normal Recalcification time 3 min 45 sec

Diagnosis *Deficiency of the antihaemophilic factor (classical haemophilia) plus deficiency* of the freezing/serum factor (Christmas factor)

Family 29

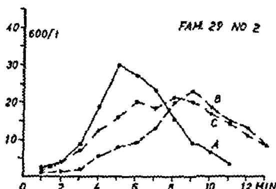


As far back as the present generation remembers *there have been no cases of* haemophilia in the family

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Blood analyses 1941 (*Andreassen*) Clotting time (Burker) 15 – > 25 min Clot loose Thrombocytes 350 000

Present investigations Quick s prothrombin time 18 19 20 sec (control 19 sec) Thrombocytes 322 000 per µl of plasma Recalcification time in dilute plasma 2 min 45 sec The thrombin generation test (Curve A) showed a perfectly normal thrombin generation with a steep rise and fall of the thrombin concentration



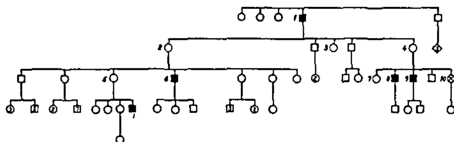
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Curve B shows the thrombin generation curve for the control plasma

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Diagnosis No detectable clotting defect

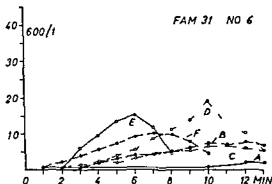
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Blood analyses Quick s prothrombin time 18 17 17 sec (control 19 sec) Thrombo cytes 17 000 (seventeen thousand) per µl of plasma Recalcification time in dilute plasma 10 min 50 sec The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma Slight improvement of the thrombin generation and a fall of the recalcification time to 4 min

Curve C Addition of normal serum Slight improvement of the thrombin generation Recalcification time 4 min 30 sec

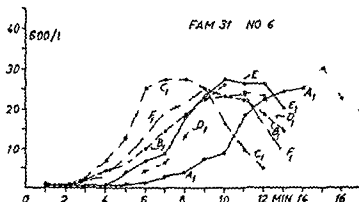
Curve D Addition of 0 4 ml of platelet suspension (120 000 platelets per µl) gave essential improvement of the thrombin generation but maximum concentration was attained fairly late Recalcification time 5 min

Curve E Simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 4 ml of platelet suspension (170 000 platelets per µl) The thrombin generation normal Recalcification time 2 min 40 sec

Curve F Simultaneous addition of 0 2 ml of serum and 0 4 ml of platelet suspension likewise resulted in a somewhat improved thrombin generation Recalcification time 2 min

Owing to the thrombopenia of which no explanation could be given the patient s blood was examined again 18 months later

Quick s prothrombin time 17 17 18 sec (control 20 sec) Thrombocytes 630 000 per µl of plasma Recalcification time in dilute plasma 6 min 50 sec The thrombin generation test showed a slow rise of the thrombin concentration after a lag period of about 8 min (Curve A_1) The maximum concentration was rather high but took 15 minutes to be attained



Curve B_1 Addition of 0 2 ml of adsorbed bovine plasma the thrombin generation was accelerated Recalcification time 3 min 30 sec

Curve C_1 Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 3 min 33 sec

Curve D_1 Addition of 0 2 ml of heated readsorbed serum The thrombin generation delayed Recalcification time 6 min

Curve E_1 Addition of the patient s own serum to his own plasma effected only slight acceleration of the thrombin generation Recalcification time 6 min

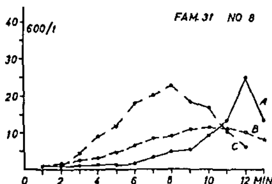
Curve F Addition of 0 5 ml of frozen platelet suspension (205 000 platelets per µl) improved the thrombin generation so much as to render it almost normal Recalcification time 4 min 45 sec

The patient s plasma was unable to normalise the thrombin generation in plasma lacking the AHF (fam 84 no 5) whereas it normalised the thrombin generation in plasma from a patient lacking the Christmas factor (fam 62 no 3)

Diagnosis Slight deficiency of the antihaemophilic factor (classical factor) Intermittent thrombopenia?

8 Born 1906 The first abnormal bleeding was observed at the age of 10–12 years when dental extraction was followed by prolonged oozing haemorrhage The patient has had numerous gingival haemorrhages since of which several required admission to hospital From the age of 13–14 there were haemarthroses most often in the knees and elbows which however never required orthopedic treatment The mobility of the right knee is slightly limited The patient has had several intramuscular bleedings some of which were localised in the throat where they caused respiratory troubles There has never been haematuria but once a gastric haemorrhage The patient is liable to post traumatic haematomas and haemarthroses He has never been incapacitated by his disease He has not been in hospital for 18 years and lives by his work as a carrier

Blood analyses Quick s prothrombin time 19 19 19 sec (control 20 sec) Thrombocytes 362 000 per µl of plasma Recalcification time 7 min 20 sec The thrombin generation test showed a greatly delayed thrombin generation (Curve A) The thrombin concentration reached high values however

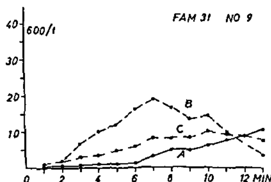


Curve B Addition of serum No improvement of the thrombin generation Recalcification time 3 min 30 sec
Curve C Addition of 0.. ml of ad orbed bovine plasma The thrombin generation normal Recalcification time 3 min

Diagnosis Deficiency of the antihaemophili factor (classical haemophilia)

9 Born 1908 The first abnormal bleeding occurred at the age of 2 years when blood continued to ooze from a wound in the forehead In infancy the patient had frequent spontaneous gingival haemorrhages From school age there were numerous haemarthroses most often in the knee joints which gradually caused permanent limitation of mobility and changes of contour There has been one episode of intra abdominal bleeding but never haematuria He has had numerous intramuscular bleedings The patient continues to have frequent haemarthroses He has not been in hospital since 1927 He is a tailor and does not feel particularly troubled by his disease

Blood analyses Quick s prothrombin time 19 19 19 sec (control 20 sec) Thrombocytes 244 000 per µl of plasma Recalcification time in dilute plasma 7 min 45 sec The thrombin generation showed negligeable thrombin generation (Curve A)



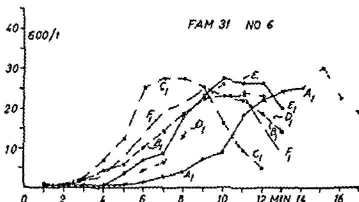
Curve B Addition of 0 ? ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 30 sec
Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 3 min 30 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Curve F Simultaneous addition of 0 2 ml of serum and 0 4 ml of platelet suspension likewise resulted in a somewhat improved thrombin generation Recalcification time 2 min

Owing to the thrombopenia of which no explanation could be given the patient's blood was examined again 18 months later

Quick's prothrombin time 17 17 18 sec (control 20 sec) Thrombocytes 630 000 per µl of plasma Recalcification time in dilute plasma 6 min 50 sec The thrombin generation test showed a slow rise of the thrombin concentration after a lag period of about 8 min (Curve A_1) The maximum concentration was rather high but took 15 minutes to be attained



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Curve D Addition of 0 2 ml of heated readsorbed serum The thrombin generation delayed Recalcification time 6 min

Curve E_1 Addition of the patient's own serum to his own plasma effected only slight acceleration of the thrombin generation Recalcification time 6 min

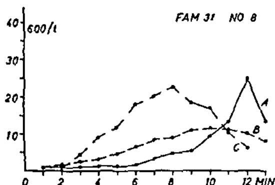
Curve F_1 Addition of 0 5 ml of frozen platelet suspension (205 000 platelets per µl) improved the thrombin generation so much as to render it almost normal Recalcification time 4 min 45 sec

The patient's plasma was unable to normalise the thrombin generation in plasma lacking the AHF (fam 84 no 5) whereas it normalised the thrombin generation in plasma from a patient lacking the Christmas factor (fam 67 no 3)

Diagnosis Slight deficiency of the antihaemophilic factor (classical factor) Intermittent thrombopenia

8 Born 1906 The first abnormal bleeding was observed at the age of 10-1 years when dental extraction was followed by prolonged oozing haemorrhage The patient has had numerous gingival haemorrhages since of which several required admission to hospital From the age of 13–14 there were haemarthroses most often in the knees and elbows which however never required orthopedic treatment The mobility of the right knee is slightly limited The patient has had several intramuscular bleedings some of which were localised in the throat where they caused respiratory troubles There has never been haematuria but once a gastric haemorrhage The patient is liable to post traumatic haematomas and haemarthroses He has never been incapacitated by his disease He has not been in hospital for 18 years and lives by his work as a carrier

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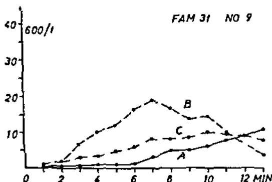
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Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

9 Born 1908 The first abnormal bleeding occurred at the age of 2 years when blood continued to ooze from a wound in the forehead In infancy the patient had frequent spontaneous gingival haemorrhages From school age there were numerous haemarthroses most often in the knee joints which gradually caused permanent limitation of mobility and changes of contour There has been one episode of intra abdominal bleeding but never haematuria He has had numerous intramuscular bleedings The patient continues to have frequent haemarthroses He has not been in hospital since 1927 He is a tailor and does not feel particularly troubled by his disease

Blood analyses Quick s prothrombin time 19 19 19 sec (control 20 sec) Thrombocytes 244 000 per μl of plasma Recalcification time in dilute plasma 7 min 45 sec The thrombin generation showed negligeable thrombin generation (Curve A)

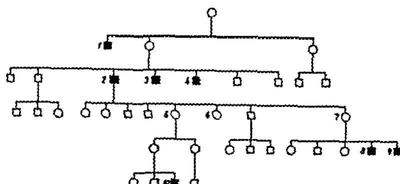


Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 30 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 3 min 30 sec

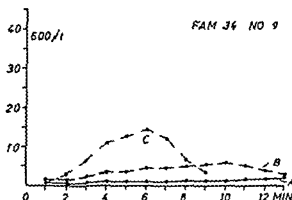
Diagnosis Defi iency of the antihaemophilic factor (cla sical haemophilia)

Family 34



9 Born 1945 There has been a tendency to subcutaneous haematomas since early infancy The patient has had several *prolonged mucosal haemorrhages after having bitten* his tongue Dentition was attended by haemorrhages which often necessitated admission to hospital He has never had haematuria melaena nor haematemesis There have been frequent haemorrhages into the knees ankles and elbows but these have not yet caused permanent changes The patient has received several blood transfusions

Blood analyses Quick's prothrombin time 18 18 17 sec (control 18 sec) Thrombocytes 570 000 per µl of plasma Recalcification time in dilute plasma 11 min The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 2 min 45 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin concentration rose and fell quickly Recalcification time 2 min 45 sec

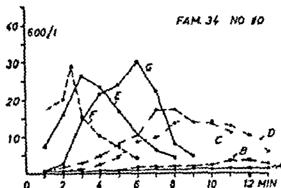
Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

10 Born 1944 The patient bruises easily and has done so from birth An attack of otitis was associated with prolonged spontaneous bleeding from the ear At the age of one year he had prolonged oozing haemorrhage from a cut in the ear He has had frequent bleedings into the elbows and knees *There is limited mobility of the right elbow X ray* of the elbows and the left leg showed the bones to be deficient in calcium Destruction and osteophyte formations were found in the elbow and knee joints as well as narrowing of the joint *cavity of the left knee The patient has been given many blood transfusions*

Blood analyses 1953 (The Rigshospitalet Dept G) Clotting time 6–45 min Capillary resistance No petechiae Prothrombin time 30 sec (control 18 sec) Bleeding time

4½ min Thrombocytes 428 000 Clotting time 1½ hours at 37 C Clotting time according to Andreassen Bürker 6–45 min

Present investigations Quick's prothrombin time 19 19 18 sec (control 20 sec) Thrombocytes 466 000 per µl of plasma Recalcification time in dilute plasma 19 min The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of serum The thrombin generation poor Recalcification time 7 min

Curve C Addition of 0 4 ml of adsorbed bovine plasma The thrombin generation normal (0 2 ml of bovine plasma was not sufficient) Recalcification time 3 min 45 sec

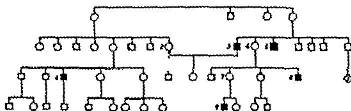
Curve D Addition of 0 2 ml of fresh plasma The thrombin generation improved but somewhat delayed *Recalcification time 4 min 45 sec*

Addition of 0 2 ml of the patient's plasma to a control plasma caused no inhibition *of the thrombin generation in the latter In other words no signs of an inhibitor were* demonstrable (Curve E illustrates the thrombin generation before addition of the patient's plasma *and curve F after)*

Curve G The patient's plasma did not lack the Christmas factor being able to normalise *the thrombin generation in plasma from a patient with Christmas disease (fam 4* no 10)

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 35



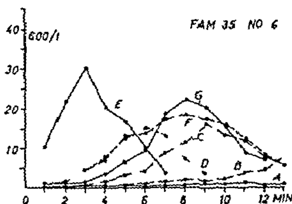
There is only one living patient with haemophilia in the family

6 Born 1906 A bleeding tendency was first noticed when the patient, at the age of 2 years had prolonged oozing haemorrhage from a minor sore in the mouth Since there have been several alveolar and gingival haemorrhages and numerous episodes of

often spontaneous epistaxis From school age there were increasingly frequent and painful haemarthroses chiefly in the knees ankles and elbows These haemorrhages have gradually caused changes of the joints X ray of the knees and elbows 1942 (The Aarhus Kommunehospital) showed narrowed joints cavities irregular sclerosed articular surfaces and pea to walnut sized subchondral areas of decreased density He has had one episode of haematuria and occasionally spontaneous intramuscular haemorrhages The patient was previously given a number of blood transfusions Though the disease has grown increasingly milder in the course of years the patient is still liable to subcutaneous haematomas and to haemarthroses The patient earns a living as a woodcutting machinist and does not receive disablement benefit

Blood analyses 1942 (The Aarhus Kommunehospital) Clotting time according to Howell Gram 11 min Bleeding time 1½ min Thrombocytes 266 000 Fibrinogen 0 51 %
Clot retraction normal

Present investigations Quick s prothrombin time 18 18 19 sec (control 16 sec) Thrombocytes 337 000 per µl of plasma Recalcification time in dilute plasma 11 min 45 sec The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of normal serum No essential improvement of the thrombin generation though the concentration rose somewhat towards the conclusion of the experiment Recalcification time 6 min 45 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma Some improvement of the thrombin generation though only after a lag period of 5 min Recalcification time 4 min 30 sec

Curve D Simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 2 ml of serum The thrombin generation normal Recalcification time 2 min 15 sec

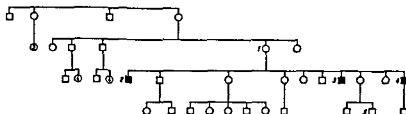
Curve E After the plasma had been stored with its normal content of platelets at −20 C for 24 hours addition of 0 2 ml of adsorbed bovine plasma caused a very steep rise and fall of the thrombin concentration Recalcification time 1 min

Curve F Use of platelet poor frozen plasma in a similar experiment gave a somewhat slower rise of the thrombin concentration Recalcification time 3 min

Curve G The thrombin generation normal after addition of 0 2 ml of adsorbed bovine plasma and 0 2 ml of heated readsorbed serum Recalcification time 4 min 10 sec

Diagnosis *Deficiency of the antihaemophilic factor plus freezing serum factor*

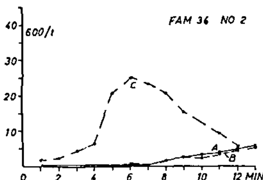
Family 36



2 Born 1913 Propositus The first abnormal bleeding manifested itself by prolonged oozing bleeding from a minor sore in the forehead when the patient was 4 years old During infancy and childhood the patient had numerous severe haemorrhages from the gums and nose In 1936 he had to have three blood transfusions to stop the bleeding after extraction of all his lower teeth From the age of 7–8 he had increasingly frequent haemarthroses chiefly in the knees and elbows where they gradually caused permanent limitation of mobility Both knees are fixed in about 35 degrees flexion There is extension defect in both elbows The patient has had several episodes of haematuria attended by lumbar pain

The patient thinks that the bleeding tendency has abated somewhat with increasing years spontaneous haemorrhages occurring less frequently than previously He is however unable to work chiefly because of his joint deformities and receives disablement benefit

Blood analyses Quick s prothrombin time 20 20 20 sec (control 20 sec) Thrombocytes 177 000 per μl of plasma Recalcification time in dilute plasma 9 min 34 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma seemed to have no influence on the thrombin generation Recalcification time 9 min 46 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 3 min 15 sec

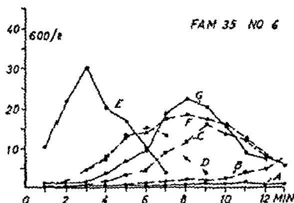
Diagnosis Christmas factor deficiency (Christmas disease)

3 Born 1920 One year old the patient had a prolonged oozing bleeding from a minor sore in the mouth Later he had numerous haemorrhages from the gums and nose and often spontaneous subcutaneous haematomas From school age there have been increasingly frequent haemarthroses chiefly in the knees shoulders and ankles The left knee joint is nearly stiff and the mobility of the right is greatly limited The left ankle is ankylotic The mobility of the elbows is greatly reduced He continues to have recurrent

often spontaneous epistaxis From school age there were increasingly frequent and painful haemarthroses chiefly in the knees ankles and elbows These haemorrhages have gradually caused changes of the joints *X ray of the knees and elbows 1942 (The Aarhus Kommunehospital)* showed narrowed joints cavities irregular sclerosed articular surfaces and pea to walnut sized subchondral areas of decreased density He has had one episode of haematuria and occasionally spontaneous intramuscular haemorrhages The patient was *previously* given a number of blood transfusions Though the disease has grown increasingly milder in the course of years the patient is still liable to subcutaneous haematomas and to haemarthroses The patient earns a living as a woodcutting machinist and does not receive disablement benefit

Blood analyses 1942 (The Aarhus Kommunehospital) Clotting time according to *Howell Gram 11 min Bleeding time 1½ min* Thrombocytes 266 000 *Fibrinogen 0 51 %* Clot retraction normal

Present investigations Quick s prothrombin time 18 18 19 sec (control 16 sec) Thrombocytes 337 000 per µl of plasma Recalcification time in dilute plasma 11 min *45 sec The thrombin generation test showed no thrombin generation (Curve A)*



Curve B Addition of 0 2 ml of normal serum No essential improvement of the thrombin generation though the concentration rose somewhat towards the conclusion of the experiment Recalcification time 6 min 45 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma Some improvement of the thrombin generation though only after a lag period of 5 min Recalcification time 4 min 30 sec

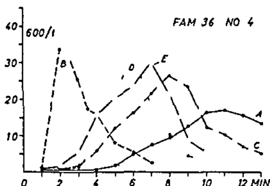
Curve D Simultaneous *addition of 0 2 ml of adsorbed* bovine plasma and 0 2 ml of serum The thrombin generation normal Recalcification time 2 min 15 sec

Curve E After the plasma had been stored with its normal content of platelets at −20 C for 24 hours addition of *0 2 ml* of adsorbed bovine *plasma caused a very steep rise and fall of the thrombin concentration* Recalcification time 1 min

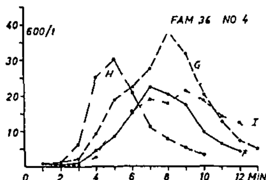
Curve F Use of platelet poor frozen plasma in a similar experiment gave a somewhat slower rise of the thrombin concentration Recalcification time 3 min

Curve G The thrombin generation normal after addition of *0 2 ml of adsorbed* bovine plasma *and* 0 2 *ml of heated readsorbed serum* Recalcification time 4 min 10 sec

Diagnosis Deficiency of the antihaemophilic factor plus freezing/serum factor



Curve B Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 1 min 40 sec
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 40 sec
Curve D Addition of 0 2 ml of readsorbed normal serum (50 mg barium sulphate) The thrombin generation normal Recalcification time 3 min 20 sec
Curve E Addition of 0 4 ml of frozen platelet suspension containing 380 000 platelets per µl of suspension The thrombin generation normal Recalcification time 3 min



Curve F Addition of heated readsorbed serum The thrombin generation normal Recalcification time 4 min
Curve G Course of the thrombin concentration in the patient s plasma after this had been stored with its normal content of platelets at −20 C for 48 hours The thrombin generation normal Recalcification time 4 min
Curve H Corresponding experiment with platelet poor plasma (9000 platelets per µl) The thrombin generation normal Recalcification time 3 min
Curve I Addition of the patient s own washed platelets (0 4 ml 130 000 platelts per µl) to the plasma from which they had been isolated The thrombin generation must be characterised as normal Recalcification time 4 min 15 sec

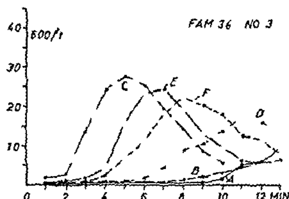
The patient s plasma normalised the thrombin generation in plasma lacking the Christmas factor (fam 85 no 1) and in plasma lacking the AHF (fam 84 no 5)

Diagnosis Deficiency of the Hageman factor (Hageman s trait)

5 Born 1955 The patient displayed no signs of a haemorrhagic diathesis till the age of about 9 months when apparently without cause he began to vomit and shortly after lost consciousness He was admitted to a neurosurgical unit where a plum sized

haematuria with attending lumbar pain He has had several episodes of intra abdominal haemorrhage with melaena The disease has not grown milder in the course of years The patient still experiences frequent apparently spontaneous intramuscular bleedings and bleedings in the right hip joint The patient receives disablement benefit

Blood analyses Quick s prothrombin time 19 19 18 sec (control 20 sec) Thrombocytes 210 000 per µl of plasma Recalcification time in dilute plasma 11 min 30 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 10 min

Curve C Addition of normal serum The thrombin generation normal Recalcification time 2 min 30 sec

Curve D Addition of 0 2 ml of frozen platelet suspension (150 000 thrombocytes per µl) Some improvement of the thrombin generation after a lag period of 6 min

Curve E The thrombin generation in platelet rich stored plasma (14 months at −20 C) was normal Recalcification time 4 min

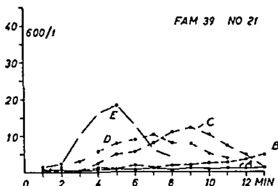
Curve F shows the result of the corresponding experiment with platelet poor plasma The thrombin generation normal Recalcifi ation time 4 min 28 sec

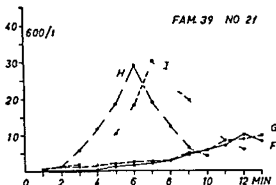
Diagnosis Christmas factor deficiency (Christmas disease)

4 Born 1928 2 years old the patient bled for a long time from a minor wound of one hand Blood transfusion was required to stop the bleeding He has had several episodes of prolonged posttraumatic gingival haemorrhage He has never had any teeth extracted From the age of 3–4 he had seven painful haemarthroses most often in the ankles The haemarthroses decreased considerably in frequency and severity after the patient had grown up There are no permanent changes of the joints In 1957 the patient was operated on for a meniscus lesion in one knee The patient was given 500 ml of plasma before the operation and during the following 24 hours three times 50 ml of plasma The next 5 days the patient received 500 ml of plasma daily There was no bleeding after the operation

The patient is a telegraph operator on board a ship and manages very well He does not receive disablement benef He is far less troubled by the disease than his two brothers with whom he has only his mother in common

Blood analyses Quick s prothrombin time 20 19 19 sec (control 19 sec) Thrombocytes 340 000 per µl of plasma Recalcification time in dilute plasma 5 min 35 sec The thrombin generation test showed a slow rise of the thrombin concentration after a lag period of 5 min (Curve A)





Curve F A corresponding experiment with platelet poor plasma (7000 platelets per μl) The thrombin generation poor Recalcification time 7 min 30 sec

Curve G Course of the thrombin generation in frozen platelet-containing plasma alone Only low thrombin concentrations were seen towards the conclusion of the experiment Recalcification time 7 min 30 sec

Curve H Signs of inhibitor were not demonstrated Addition of 0 2 ml of normal fresh plasma resulted in a normal thrombin generation

Curve I Simultaneous addition of 0 2 ml of bovine plasma and 0 2 ml of heated read sorbed serum to stored platelet poor plasma gave a normal thrombin generation Recal cification time 4 min 10 sec

The patient s frozen plasma normalised the thrombin generation in plasma lacking the Christmas factor from pt no 1 fam 85 but only partially so in plasma from pt no 10 family 4

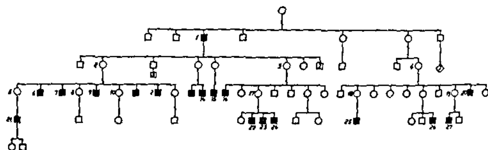
Diagnosis Deficiency of the antihaemophilic factor plus freezing/serum factor

23 Born 1941 From the age of 10 months the patient had subcutaneous haemorrhages after minor injuries At the age of 2 years there was prolonged bleeding from the gum after an injury The second dentition was associated with profuse bleedings Injections give rise to haematomas There have been frequent bleedings into the knees and ankles The mobility of both knees is greatly limited In 1955 the patient had an intrapericardial haemorrhage after injury of the thoracic wall The patient has received several transfusions

Blood analyses (The Vejle Amts og Bys Sygehus) 1955 Clotting time 40 min Bleeding time $10^1{}_2$ min Thrombocytes 330 000

haematoma was found in the right hemisphere The patient died few hours after the operation Autopsy revealed a large up to 3 cm thick subarachnoid haematoma in the right fissure of Sylvius extending along the lateral and inferior surfaces of the entire right temporal lobe The patient was given transfusions during and after the operation 6 hours after death blood was withdrawn for analysis The thrombin generation was somewhat slow The recalcification time exceeded 24 minutes No definite conclusions can be drawn on the basis of this information

Family 39



21 Born 1922 A bleeding tendency first manifested itself at the age of about one year In infancy the patient most often had bleedings into the skin and from the gums more rarely from the nose From the age of 3 years there were increasingly frequent and painful haemarthroses comprising nearly all the large joints chiefly the knees the mobility of which has gradually become limited with attending pronounced muscular atrophy Both elbows present a slight extension defect Since the age of 5–6 years the patient has had several episodes of haematuria At the age of 12 he had profuse intra muscular haemorrhage round the left hip and extending into the pelvis The haemorrhage caused prolonged loss of sensation in the anterior part of the thigh At the age of 15 he had another haemorrhage in the same region This also gave loss of sensation in the anterior part of the thigh as well as paralysis of the extensors of the thigh These neurologic symptoms persisted for 2 years The patient has had several episodes of melaena He has extracted some of his own teeth without this having been followed by abnormal bleeding The disease has grown milder since the age of 20 Formerly the patient could only get about in a wheeled chair but now he is able to ride on a bicycle The patient live by teaching music and has a small shop

Blood analyses Quick s prothrombin time 16 17 16 sec (control 16 sec) Thrombocytes 233 000 per µl of plasma Recalcification time in dilute plasma 12 min 40 sec The thrombin generation test showed no thrombin generation (Curve A)

Curve B Addition of 0 2 ml of serum No essential improvement of the thrombin generation Recalcification time 6 min

Curve C Addition of 0 2 ml of adsorbed bovine plasma A slow rise of the thrombin concentration and a somewhat more rapid fall Recalcification time 4 min

Curve D Addition of twice as much adsorbed bovine plasma Only little further improvement of the thrombin generation

Curve E Addition of 0 2 ml of adsorbed bovine plasma to the patient s plasma with its normal content of platelets after this had been stored at –20 C for 27 days gave a normal thrombin generation Recalcification time 2 min 15 sec

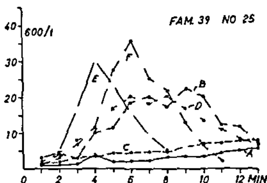
Curve B Addition of 0 2 ml of serum Very little improvement of the thrombin generation which was poor Recalcification time 5 min 30 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recal ification time 3 min 19 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

25 Born 1925 One year old the patient had his first abnormal bleeding In infancy he most often had mucosal bleedings specially from the gums Some of these were life threatening Subcutaneous haemorrhages were likewise frequent From the age of 2–3 years he had increasingly frequent and painful haemarthroses gradually comprising nearly all the large joints chiefly however the knees elbows and ankles resulting in transitorily limited mobility He has had several intra abdominal bleedings accompanied by haematemesis and numerous intramuscular bleedings In the left hand such bleeding caused development of ulnar paralysis resulting in claw hand and loss of sensation in the skin These neurologic phenomena persist The patient has never been troubled by epistaxis He states that the disease has grown milder since the age of 21 He occasionally has haemarthroses but these are less pronounced than previously The patient is fully fit for his work as a provision dealer He has never been given transfusions

Blood analyses Quick s prothrombin time 20 21 20 sec (control 18 sec) Thrombocytes 237 000 per μl of plasma Recalcification time in dilute plasma 9 min The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 40 sec

Curve C Addition of 0 2 ml of serum The thrombin generation poor Recalcification time 3 min 12 sec

Curve D Addition of 0 2 ml of fresh platelet suspension (?00 000 per μl) and 0 2 ml of adsorbed bovine plasma did not accelerate the thrombin generation more than did bovine plasma alone

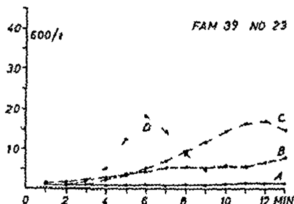
Curve E Addition of 0 2 ml of adsorbed bovine plasma to the patient s plasma after this had been stored with its normal content of platelets at −20 C for 7 months effected a more rapid rise and fall of the thrombin concentration than when the adsorbed bovine plasma was added to fresh plasma (curve B) Recalcification time 2 min 30 sec

Curve F Simultaneous addition of 0 2 ml of heated readsorbed serum likewise gave considerable acceleration of the thrombin generation Recal ification time 3 min

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

26 Born 1949 The patient has bruised easily since the age of 2–3 months He has been in hospital several times on account of epistaxis and prolonged bleedings from

Present investigations Quick s prothrombin time 18 18 18 sec (control 18 sec) Thrombocytes 800 000 per µl of plasma Recalcification time in dilute plasma 20 min The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of *serum* Little improvement of the thrombin generation Recalcification time 5 min 45 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma Very slow rise of the thrombin concentration Recalcification time 4 min 20 sec

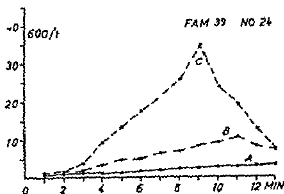
Curve D Addition of 0 2 ml of adsorbed bovine plasma to the patient s platelet poor plasma (8000 platelets per µl of suspension) after this had been stored at −20 C for 10 months effected normal thrombin generation Recalcification time 3 min 21 sec

Diagnosis Deficiency of the antihaemophilic factor plus freezing/serum factor

24 Born 1945 The patient has had abnormal bleeding phenomena from the age of 6 months There were prolonged bleedings on first dentition Injury of the gum was followed by prolonged bleeding He has had several haemorrhages into the left ankle but these have not left permanent changes There has been neither haematemesis nor melaena but haematuria The patient has been given several transfusions with favourable results

Blood analyses (The Vejle Amts og Bys Sygehus Med Dept) *Clotting time 45 min 50 min* Bleeding time 3 min Thrombocytes *489 000*

Present investigations Quick s prothrombin time *18 19 18 sec (control 18 sec)* Thrombocytes 542 000 *per µl of plasma Recalcification time in dilute plasma 12 min 30 sec The thrombin generation test showed negligeable thrombin generation (Curve A)*



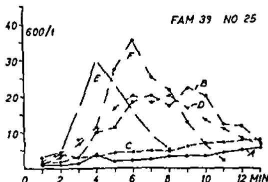
Curve B Addition of 0 2 ml of serum Very little improvement of the thrombin generation which was poor Recalcification time 5 min 30 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 19 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

25 Born 1925 One year old the patient had his first abnormal bleeding In infancy he most often had mucosal bleedings specially from the gums Some of these were life threatening Subcutaneous haemorrhages were likewise frequent From the age of 2–3 years he had increasingly frequent and painful haemarthroses gradually comprising nearly all the large joints chiefly however the knees elbows and ankles resulting in transitorily limited mobility He has had several intra abdominal bleedings accompanied by haematemesis and numerous intramuscular bleedings In the left hand such bleeding caused development of ulnar paralysis resulting in claw hand and loss of sensation in the skin These neurologic phenomena persist The patient has never been troubled by epistaxis He states that the disease has grown milder since the age of 21 He occasionally has haemarthroses but these are less pronounced than previously The patient is fully fit for his work as a provision dealer He has never been given transfusions

Blood analyses Quick s prothrombin time 20 21 20 sec (control 18 sec) Thrombocytes 237 000 per μl of plasma Recalcification time in dilute plasma 9 min The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 40 sec

Curve C Addition of 0 2 ml of serum The thrombin generation poor Recalcification time 3 min 12 sec

Curve D Addition of 0 2 ml of fresh platelet suspension (00 000 per μl) and 0 2 ml of adsorbed bovine plasma did not accelerate the thrombin generation more than did bovine plasma alone

Curve E Addition of 0 ml of adsorbed bovine plasma to the patient s plasma after this had been stored with its normal content of platelets at −20 C for 7 months effected a more rapid rise and fall of the thrombin concentration than when the adsorbed bovine plasma was added to fresh plasma (curve B) Recalcification time min 30 sec

Curve F Simultaneous addition of 0 2 ml of heated readsorbed serum likewise gave considerable acceleration of the thrombin generation Recalcification time 3 min

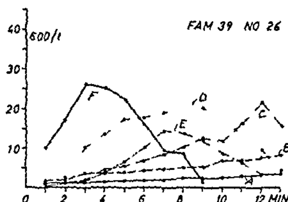
Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

26 Born 1949 The patient has bruised easily since the age of 2–3 months He has been in hospital several times on account of epistaxis and prolonged bleedings from

wounds The patient has received several transfusions He has had haemorrhages into the elbows ankles and knees but these have left no permanent changes

Blood analyses (The Vejle Amts og Bys Sygehus Med Dept) Clotting time 3? min – 2 hours Bleeding time 15 min 3 min Thrombocytes 312 000

Present investigations Quick s prothrombin time 19 19 19 sec (control 19 sec) Recalcification time in dilute plasma 8 min 30 sec The thrombin generation test showed *negligeable thrombin generation* (Curve A)



Curve B Addition of 0 2 ml of normal serum No essential improvement of the thrombin generation Recalcification time 4 min

Curve C Addition of 0 2 ml of adsorbed bovine plasma Slow rise of the thrombin concentration Recalcification time 4 min

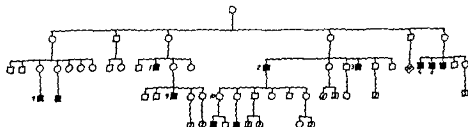
Curve D Addition of 0 2 ml of adsorbed bovine plasma to the patient s plasma after this had been stored with it normal content of platelets at –20 C for 24 hours The thrombin generation normal Recalcification time 3 min

Curve E shows the result of the same experiment with frozen platelet poor plasma The thrombin generation essentially improved but the concentrations attain d were not particularly high Recalcification time 4 min

Curve F Simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 2 ml of serum The thrombin generation normal Recalcification time 1 min 45 sec

Diagnosis Deficiency of the antihaemophilic factor plus freezing serum factor

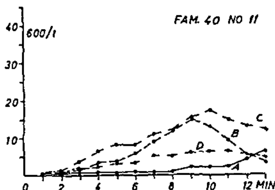
Family 40



11 Born 1927 Propositus The patient is severely affected by haemophilia Since the age of 3 years he has had frequent mucosal haemorrhages specially oozing gingival bleedings several of which were dangerous and necessitated admission to hospital Later he experienced painful often spontaneous haemarthroses comprising all the large joints

Su h have caused limited mobility of the right knee and the left elbow He has had numerous intramuscular bleedings which together with the haemarthroses have kept the patient bedridden for long periods from his 8th year He has had many episodes of *haematuria and frequent* subcutaneous haematomas The abnormal bleeding phenomena have not abated with increasing years The patient is greatly disabled He receives disablement benefit but supplements his income by dealing a little with motor-cars The patient has received many transfusions

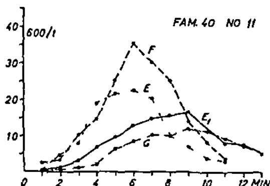
Blood analyses Quick s prothrombin time 18 19 19 sec (control 19 sec) Thrombocytes 490 000 per µl of plasma Recalcification time in dilute plasma 10 min 30 sec The thrombin generation test showed negligeable thrombin generation (Curve *A*)



Curve B Addition of 0 2 ml of adsorbed bovine plasma Some improvement of the thrombin generation which however continued to be greatly delayed Recalcification time 4 min 15 sec

Curve C Addition of 0 4 ml of adsorbed bovine plasma No further improvement of the thrombin generation

Curve D Addition of 0 2 ml of normal serum Slight improvement of the thrombin generation Recalcification time 3 min 45 sec



Curve E Addition of 0 2 ml of adsorbed bovine plasma plus 0 2 ml of normal serum The thrombin generation normal Recalcification time 3 min

Curve E1 Using readsorbed heated serum instead of serum in the above experiment (E) the rise of the thrombin concentration was lower and very slow

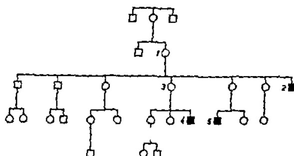
Curve F *No signs of an inhibitor Addition of 0 2 ml of* citrated plasma (fresh) to 1 ml of the patient s plasma gave a steep rise and fall of the thrombin concentration Recalcification time 2 min 45 sec

Curve G Addition of 0 2 ml of adsorbed bovine plasma to the patient s plasma after this had been stored with its normal content of platelets at -20 for 18 days effected only slight improvement of the thrombin generation Recalcification time 4 min 30 sec

After having been stored at -20 C the patient s plasma normalised the thrombin generation in plasma lacking the Christmas factor (fams 4 14 and 22)

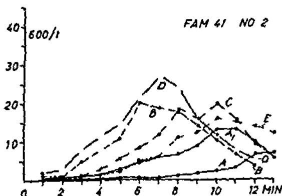
Diagnosis Deficiency of the antihaemophilic factor and of the Christmas factor

Family 41



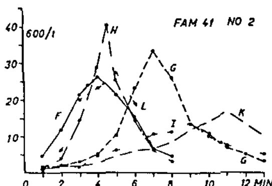
The family is known as far back as before 1840 No cases of haemophilia are known beyond those described below

2 Born 1921 Propositus A bleeding tendency was noticed from the patient s first year of life In infancy he had prolonged profuse often spontaneous mucosal haemorrhages and bleedings into the skin From the age of 5-6 he had increasingly frequent painful haemorrhages into nearly all the joints knees ankles elbows wrists and hips Such gradually gave limited mobility of the elbows and knees The patient has had a few episodes of haematuria attended by unilateral lumbar pain In 1941 he had a very severe attack of haematuria accompanied by a very large haematoma in the right flank He has had a few episodes of intra abdominal haemorrhage associated with haematemesis Epistaxis was frequent previously The patient has had several gingival haemorrhages and prolonged bleedings after dental extraction which latter necessitated transfusion The patient has been given numerous transfusions allegedly with a favourable effect The disease has remained unchanged in the course of years The patient is totally disabled nearly always troubled by bleeding somewhere or other He receives disablement benefit The patient has been trained as a shoemaker but is unable to work as such



Blood analyses Quick s prothrombin time 18 18 17 sec (control 19 sec) Thrombocytes 369 000 per µl of plasma Recalcification time in dilute plasma 8 min 25 sec The thrombin generation test showed negligeable thrombin generation (Curve A) The curve marked A_1 shows the course of the thrombin concentration in a blood sample withdrawn 10 months later The recalcification time was then 5 min 50 sec

Curve B The thrombin generation normal after addition of 0 2 ml of adsorbed bovine plasma Recalcification time 3 min 40 sec
Curve C Addition of 0 2 ml of normal serum The thrombin generation considerably improved though with a slightly prolonged lag period and a somewhat late occurrence of maximum thrombin concentration Recalcification time 4 min 30 sec
Curve D Addition of 0 2 ml of readsorbed normal serum The thrombin generation normal Recalcification time 3 min 45 sec
Curve E Addition of 0 2 ml of heated readsorbed normal serum Some improvement of the thrombin generation Recalcification time 7 min



Curve F Simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 2 ml of heated readsorbed normal serum The thrombin generation normal Recalcification time 1 min 30 sec
Curve G Addition of 0 2 ml of the patient s own serum after this had been stored for 10 months The thrombin generation normal the lag period about 4 min Recalcification time 5 min 30 sec
Curve H The thrombin generation normal after addition of 0 2 ml of adsorbed bovine plasma to the patient s plasma (5000 platelets per µl) after this had been stored at −20 C for 3 days Recalcification time 2 min 28 sec
Curve I The thrombin generation in plasma with its normal content of platelets after storage at −20 C Little improvement The recalcification time had fallen to 4 min
Curve K 0 2 ml of the patient s plasma improved the thrombin generation somewhat in plasma from a patient with classical haemophilia (fam 84 no 5) Recalcification time 5 min 20 sec
Curve L Addition of the patient s frozen plasma to plasma from a patient with Christmas disease (fam 85) normalised the thrombin generation Recalcification time 2 min 25 sec

Diagnosis Slight deficiency of the antihaemophilic factor plus freezing/serum factor

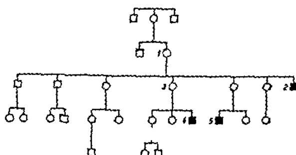
4 Born 1939 The patient displayed a bleeding tendency from the age of 6 months manifesting itself by large often spontaneous haematomas on the body as well as prolonged oozing haemorrhages from the gums The patient died 7 years old at home from haemophilia

Curve G Addition of 0 2 ml of adsorbed bovine plasma to the patient s plasma after this had been stored with its normal content of platelets at -20 for 18 days effected only slight improvement of the thrombin generation Recalcification time 4 min 30 sec

After having been stored at -20 C the patient s plasma normalised the thrombin generation in plasma lacking the Christmas factor (fams 4 14 and 22)

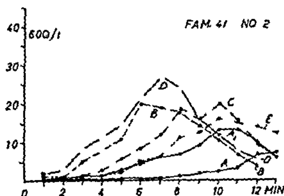
Diagnosis Deficiency of the antihaemophilic factor and of the Christmas factor

Family 41



The family is known as far back as before 1840 No cases of haemophilia are known beyond those described below

2 Born 1921 Propositus A bleeding tendency was noticed from the patient s first year of life *In infancy he had prolonged profuse often spontaneous mucosal haemo* rrhages and bleedings into the skin From the age of 5-6 he had increasingly frequent painful haemorrhages into nearly all the joints knees ankles elbows wrists and hips Such gradually gave limited mobility of the elbows and knees The patient has had a few episodes of haematuria attended by unilateral lumbar pain In 1941 he had a very severe attack of haematuria *accompanied by a very large haematoma in the right flank* He has had a few episodes of intra abdominal haemorrhage associated with haematemesis *Epistaxis was frequent previously The patient has had several gingival haemorrhages and* prolonged bleedings after dental extraction which latter necessitated transfusion The pa tient has been given numerous transfusions allegedly with a favourable effect The dis ease has remained unchanged in the course of years The patient is totally disabled nearly always troubled by bleeding somewhere or other He receives disablement benefit The patient has been trained as a shoemaker but is unable to work as such



Curve H Addition of 0 2 ml of heated readsorbed serum The thrombin generation poor Recalcification time 7 min 40 sec

Curve I Simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 2 ml of heated readsorbed serum The thrombin generation normal Recalcification time 2 min 15 sec

Curve K Addition of 0 2 ml of the patient s own serum after this had been stored for 10 months A slow but considerable rise of the thrombin concentration Recalcification time 4 min

Curve L Addition of 0 2 ml of adsorbed bovine plasma to the patient s plasma after this had been stored at −20 C for 3 days The thrombin generation normal Recal cification time 3 min 33 sec

The patient s plasma behaved like that of patient no 2 in the presence of plasma lacking the AHF and plasma lacking the Christmas factor

Diagnosis Slight deficiency of the antihaemophilic factor plus freezing/serum factor

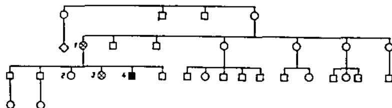
Comments

It was difficult to classify the patients in family 41 They differ from the remaining patients belonging to the group of AHF plus freezing/serum factor deficiency in that the thrombin generation in their plasma became normal after addition of adsorbed bovine plasma alone However when the plasma had been frozen the thrombin generation was accelerated very considerably after addition of adsorbed bovine plasma The same reac tion was observed after simultaneous addition of adsorbed bovine plasma and heated readsorbed serum to the fresh plasma Addition of normal serum alone gave almost normal thrombin generation The same effect was obtained by adding the patient s own serum

It seems as if addition of merely one of the two factors these patients are supposed to lack is able to normalise the coagulation system

That the patients were not referable to the group of PTA deficiency appears further from the fact that their plasma did not correct the clotting defect in plasma lacking the AHF

Family 42

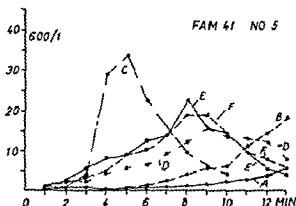


4 Born 1927 Propositus A bleeding tendency was manifest from the age of 6 months In infancy the patient had many prolonged often life threatening haemorrhages from the gums and nose which necessitated blood transfusions From the age of 2 years painful haemarthroses were very frequent gradually comprising nearly all the large joints The mobility of the knees elbows and ankles has become permanently limited The ri ht knee is ankylosed and there is pronounced muscular atrophy of the entire right lower leg The patient has for several years been wearing a plaster or leather bandage round the right leg He has had several episodes of haematuria accompanied by lumber pain The patient is greatly disabled The disease persists unchanged

Blood analyses 1941 (The Københavns Amtssygehus Gentofte) Clotting time accord ing to Howell Gram 20 min

5 Born 1937 A bleeding tendency was first noticed immediately after birth when the patient presented large ecchymoses He has had several episodes of epistaxis and bleedings from the gums *There has been no haematuria nor bleeding from the gastro-intestinal tract* He has had haemarthroses comprising most of the large joints knees ankles elbows wrists hips The right elbow is fixed in extension position The patient s disease persists unchanged *He* is disabled and receives disablement benefit

Blood analyse Quick s prothrombin time 19 19 20 sec (control 19 sec) Thrombocytes 165 000 per µl of plasma Recalcification time in dilute plasma 8 min 40 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



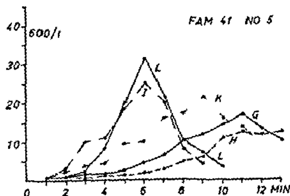
Curve B After storage of the *patient s plasma with its normal content of platelets* at −20 C for 5 months a lag period of 5–7 minutes was followed by some rise of the thrombin concentration Recalcification time 7 min 46 sec

Curve C Addition of 0 2 ml of fresh control plasma The thrombin generation *normal* Recalcification time 3 min 20 sec

Curve D The thrombin generation was somewhat better in the patient s fresh plasma about 10 months after the experiment illustrated in curve A Recalcification time *4 min 50 sec*

Curve E Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation essentially improved but the thrombin concentration rose fairly slowly Recalcification *time 2 min 45* sec

Curve F Addition of 0 2 ml of normal serum A slow rise of the thrombin concentration Recalcification time 4 min 20 sec



Curve G Addition of readsorbed serum The thrombin generation poor Recalcification time 5 min 57 se^

Curve B_1 Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 1 min 48 sec

Curve C_1 Addition of 0 2 ml of normal serum The thrombin generation considerably improved but not normal Recalcification time 4 min

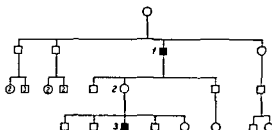
Comments

The results of these latter investigations suggest that the patient has an isolated AHF deficiency since bovine plasma alone normalised the thrombin generation On the other hand addition of serum seemed to have some influence on the thrombin generation appreciable amounts of thrombin having been formed The possibility might be conceived that the deficiency of the AHF and the Christmas factor is slight so that adsorbed bovine plasma *which perhaps is not quite without Christmas factor is able to normalise* the thrombin generation

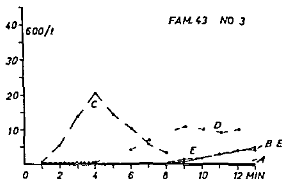
It is remarkable that the recalcification time was nearly twice as long on the first investigation (15 min) as on the second The same was true for the clotting time t of the fibrinogen samples in the thrombin generation test. These facts suggest that the clotting defect is not constant

Diagnosis (first investigation) Deficiency of the antihaemophilic factor and the Christmas factor

Family 43

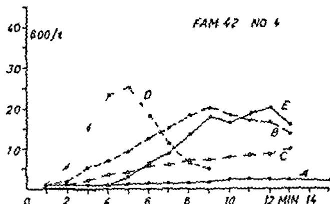


3 Born 1936 Propositus A bleeding tendency was first observed when the patient at the age of 6 months developed large apparently spontaneous haematomas round both eyes Since there have been numerous gingival haemorrhages and especially prolonged oozing often life threatening bleedings from the nose Such have necessitated admission to hospital eight times The patient has received four blood transfusions owing to loss of blood from epistaxis Since the age of 2 years the patient has had increasingly frequent haemarthroses in nearly all the large joints The *haemarthroses most often come on*



1942 Clotting time 30½–35 *min* *Clot loose* *Blood platelets* *241 000* *Blood group A*

Present investigations Spontaneous clotting time for whole blood at 37 C 68 min Quick s prothrombin time 21 19 19 sec (control 19 sec) Thrombocytes 313 000 per µl Recalcification time in dilute plasma 15 min The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 3 ml of adsorbed bovine plasma gave some improvement of the thrombin generation the thrombin concentration rising very slowly The inactivation *of the thrombin formed likewise proceeded very slowly Recalcification time 3 min 15 sec Addition of 0 5 ml of adsorbed bovine plasma did not normalise the thrombin* generation either

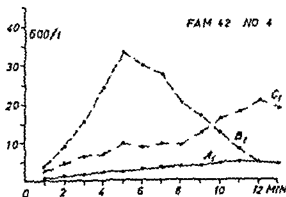
Curve C Addition of 0 2 ml of normal serum Very slight improvement of the thrombin generation Recalcification time 5 min

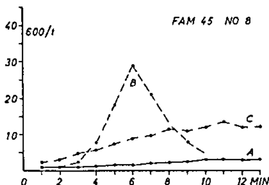
Curve D Addition of 0 2 ml of adsorbed bovine plasma as well as 0 2 ml of normal serum effected a steep rise and fall of the thrombin concentration Recalcification *time 2 min 33* sec

Curve E Addition of 0 2 ml of readsorbed serum had hardly any effect not even when added simultaneously with 0 2 ml of adsorbed bovine plasma Recalcification time 5 min 23 sec

The thrombin generation was normalised neither by plasma lacking the AHF (fam 2 no *11)* nor by Christmas plasma (fam 23 no 3)

2 years after the above investigation the patient s blood was examined again Quick s prothrombin time 20 20 20 sec (control 19 sec) Thrombocytes 366 000 per µl of plasma Clotting time for whole blood at 37 C 1 hour Recalcification time in dilute plasma 7 min The thrombin generation test showed negligeable thrombin generation (Curve A1)





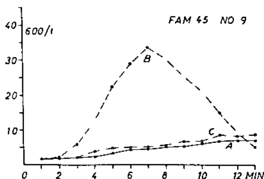
Curve B Addition of 0 ? ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 15 sec

Curve C Addition of 0 ? ml of normal serum effected a very slow rise of the thrombin concentration which however did not attain high values Recalcification time 3 min

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

9 Born 1923 A bleeding tendency was first observed when the patient was 1 month old In infancy he had numerous severe bleedings from the gums and nose which often necessitated admission to hospital and required blood transfusions From the age of 3 years he had increasingly frequent haemarthroses gradually comprising nearly all the large joints These caused greatly limited mobility of the knees elbows and ankles The haemarthroses increased very considerably in frequency with increasing years During one period the liability to such was so pronounced that bleeding into the joints might occur merely by the patient getting out of bed Between the ages of 17 and 19 the patient had about 50 haemarthroses a year He has had numerous episodes of haematuria accompanied by unilateral lumbar pain and several severe intra abdominal haemorrhages attended by haematemesis and ileus like states He has had several intramuscular bleedings of which a few were localised in the throat where they caused respiratory troubles The patient feels greatly handi apped by his disease but is inclined to think that it has grown somewhat milder since the age of about 21 He has often been bedridden for about 6 months at a time He has been given numerous transfusions with a favourable effect The patient receives disablement benefit which he supplements by doing some office work

Blood analyses Quick s prothrombin time 18 16 17 sec (control 18 sec) Thrombocytes 367 000 per µl of plasma Recalcification time in dilute plasma 9 min 16 sec The thrombin generation showed negligeable thrombin generation (Curve A)



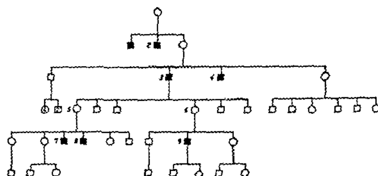
spontaneously They have caused permanent limitation of mobility in the knees and elbows At the time of investigation there were bleedings into the shoulder and neck muscles The patient has had *several episodes of haematuria with attending lumbar pain* as well as of melaena He is disabled and receives disablement benefit

Blood analyses Quick s prothrombin time 21 22 21 sec (control 19 sec) Thrombocytes 122 000 per µl Recalcification time in dilute plasma 18 min The thrombin *generation test showed* no thrombin generation (Curve A)

Curve B Addition of adsorbed bovine plasma The thrombin generation poor Recalcification time 9 min
Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recal*cification time 2 min 15 sec*
Curve D Addition of 0 2 ml of *heated* readsorbed serum *The thrombin generation* poor though slightly improved Recalcification time 5 min 45 sec
Curve E The thrombin generation improved relatively little after the patient s plasma had been stored with its normal platelet content at −20 C for 7 months Recalcification time *10 min*

Diagnosis Christmas disease

Family 45



8 Born *1925* At *the* age of *2* months the patient got large *apparently spontaneous haematomas on the arms and legs From the* age of 2 years he has had very severe often life threatening gingival haemorrhages and from the age of 5–6 years increasingly frequent haemarthroses chiefly in the right knee shoulders elbows and ankles The mobility of the knees and elbows *has gradually become permanently limited He has had haematuria twice* each *time* lasting one month There has been prolonged bleeding after dental extraction The patient thinks that the bleeding phenomena tend to occur intermittently and that they have abated somewhat since he has grown up He has received a single blood transfusion *The patient has tried to work at a radio works but he gets bleedings in the shoulder joints on the* least *physical* exertion He receives disablement benefit

Blood analyses Quick s prothrombin time 18 18 18 sec (control 18 sec) Thrombocytes 156 000 per µl of plasma *Recalcification time in dilute plasma 12 min 30 sec* The *thrombin generation* test showed negligeable thrombin generation (Curve A)

Present investigations Clotting time according to Bürker Andreassen 11 min–12½ min Quick s prothrombin time 19 19 19 sec (control 19 sec) Thrombocytes 302 000 per μl of plasma Recalcification time in dilute plasma 5 min 50 sec The thrombin generation test showed a very slow rise of the thrombin concentration (Curve A)

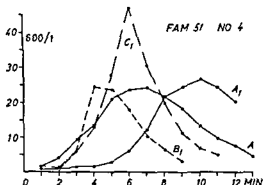
Curve B Addition of 0 2 ml of adsorbed bovine plasma No essential improvement of the thrombin generation Recalcification time 3 min 30 sec
Curve C Addition of 0 2 ml of serum The thrombin generation normal Recalcification time 1 min
Curve D Freezing of the plasma gave no unquestionable improvement of the thrombin generation Recalcification time 4 min 30 sec
Curve E Addition to this frozen plasma of 0 2 ml of platelet suspension (150 000 thrombocytes per μl) effected a rapid rise and fall of the thrombin concentration Recalcification time 3 min
Curve F Addition of heated readsorbed serum Some improvement of the thrombin generation but the lag period was over 4 min Recalcification time 5 min 15 sec

Diagnosis Christmas factor deficiency (Christmas disease)

4 Born 1933 One year old the patient was operated on for incarcerated hernia without extraordinary bleeding A bleeding tendency first manifested itself when the patient was 2 years old as frequent episodes of epistaxis and bleedings into the skin At the age of 7 he had a prolonged oozing haemorrhage following extraction of two teeth At the age of 15 there was again prolonged bleeding after dental extraction 13 years old he experienced bleeding into the left knee but he has had no other haemarthroses There has never been haematemesis haematuria nor melaena The past 6 years the patient has had no abnormal bleeding phenomena

Blood analyses 1943 *(Andreassen)* Clotting time 12–15 min

Present investigations Clotting time according to Burker Andreassen 11–12½ min Clotting time for whole blood at room temperature (25 C) 16 min Quick s prothrombin time 20 20 20 sec (control 18 sec) The thrombin generation test showed a normal thrombin generation (Curve A) Recalcifi ation time 2 min 50 sec As a reduced thrombo



plastic activity was not demonstrable in the patient s plasma the investigation was repeated 8 days later Quick s prothrombin time 18 18 18 sec (control 18 sec) The thrombin generation test again showed a normal thrombin generation Recalcification time 3 min 50 sec

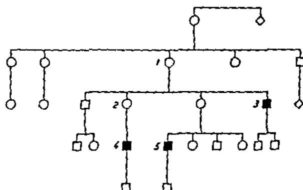
The patient s plasma normalised the thrombin generation in plasma from a patient lacking the Christmas factor (fam 4 no 10) and from a patient with AHF deficiency (fam 2 no 16) The recalcification times were 3 min 30 sec and 3 min 5 sec respectively

In 1958 the patient appeared however with a desire for a certificate of freedom from haemophilia A renewed blood analysis revealed pathological conditions

Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min
Curve C Addition of 0 2 ml normal serum The thrombin generation poor Recalcification time 6 min

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

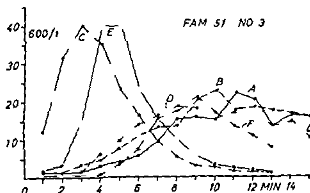
Family 51

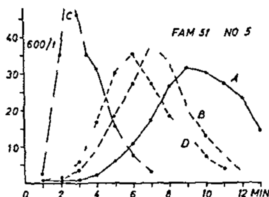


A fairly good knowledge is had of the family through several generations No cases of haemophilia are traceable in previous generations

3 Born 1919 Propositus From his first year of life the patient was liable to episodes of profuse epistaxis partly spontaneous and partly after even the slightest injuries In infancy he also frequently had large subcutaneous haematomas 11 years old he had a bleeding into one knee At the age of 12 another haemarthrosis in the same joint Though the latter was of short duration the blood persisted for 2 months At the age of 14 the patient was submitted to adenotomy This gave rise to prolonged oozing haemorrhage which did not stop till the patient had received a transfusion A subsequent dental extraction was likewise followed by oozing haemorrhage of 3 weeks duration This necessitated another blood transfusion The patient bruises easily He is a mechanic and manages his work without difficulty He is able to support his family He has done his military service

Blood analyses 1941 (The Rigshospitalet Med Out Patient Dept) Howell Gram 1) 6 min 2) 4 min 30 sec Blood platelets 298 000 Bleeding time 7 min Blood group 0
1942 *(Andreassen)* Clotting time 9–14½ min Clot loose Bleeding time ½ min

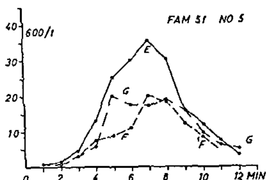




Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 35 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 1 min 19 sec

Curve D Addition of 0 2 ml of heated readsorbed normal serum The thrombin generation normal Recalcification time 3 min 26 sec



Curve E Addition of 0 2 ml of frozen platelet suspension (178 000 platelets per µl) The thrombin generation normal Recalcification time 3 min 36 sec

Curve F Course of the thrombin generation in the patient s plasma after this had been stored with its normal content of platelets at −20 C for 2 months The rise and the fall of the thrombin concentration were here steeper than in curve A Recalcification time 3 min 40 sec

The patient s plasma normalised the thrombin generation in plasma from a patient with Christmas disease (fam 85 no 1)

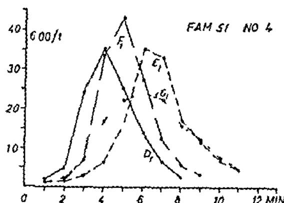
An analysis of the patient s blood 3 months later at a time when he had no bleeding phenomena the recalcification time was found to be 3 min 20 sec Quick s prothrombin time 19 19 19 sec (control 18 sec) Thrombocytes 423 000 per µl of plasma The thrombin generation was normal (Curve G)

Diagnosis Deficiency of the Hageman factor

Quick s prothrombin time 18 18 18 sec (control 17 sec) Thrombocytes 642 000 per µl of plasma Recalcification time in dilute plasma 5 min 50 sec The thrombin generation test showed considerable thrombin generation but after a lag period prolonged to a scant 6 min (Curve A₁)

Curve B₁ Addition of heated readsorbed serum The thrombin generation normal Recalcification time 2 min 45 sec

Curve C₁ Addition of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 50 sec



Curve D₁ Addition of the patient s own washed platelets to the plasma from which they had been isolated The thrombin generation normal Recalcification time 2 min 30 sec

Curve E₁ Addition of plasma from a patient with AHF deficiency (fam 84 no 5) The thrombin generation normal Recalcification time 4 min 45 sec

Curve F Addition of plasma from a patient with Christmas disease (fam 14) The thrombin generation normal Recalcification time 3 min 10 sec

Curve G Addition of serum from the same Christmas patient The thrombin generation normal Recalcification time 2 min 50 sec

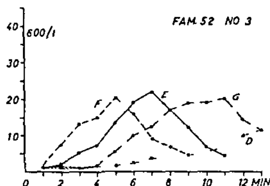
The thrombin generation was normal in the patient s platelet poor plasma (3000 platelets per µl) after this had been stored at −20 C

Diagnosis Deficiency of the Hageman factor

5 Born 1931 The patient was not aware of a bleeding tendency until the age of 26 He has never had haemarthroses epistaxis haematuria melaena haematemesis nor bleedings into the skin The patient had previously had a tooth extracted without this having been followed by secondary bleeding At the age of 26 he had two teeth extracted on account of a dental abscess This was followed 12 hours later by oozing haemorrhage After tamponade there was haemostasis for 2–3 days but then the bleeding recurred and persisted for 9 days The bleeding was so pronounced that the patient was admitted to hospital in a shocked state with a haemoglobin level of 30% He was unconscious 2 litres of blood were required to procure haemostasis There were no large bleeding vessels

Blood analyses (The Rigshospitalet Dept A) Thrombocytes 397 000 Bleeding time 1 min 45 sec Clotting time 4 min 15 sec (after transfusion) Prothrombin 95%–113%

Present investigations 2 days after the above mentioned blood transfusion Quick s prothrombin time 17 17 17 sec (control 17 sec) Thrombocytes 620 000 per µl of plasma Recalcification time in dilute plasma 4 min 25 sec The thrombin generation test showed high thrombin concentrations but the concentration did not rise till after a lag period of *4 min The inactivation of the thrombin was not concluded after 13 min* (Curve A)



Curve F Addition of 0 2 ml of adsorbed bovine plasma alone to the patient s plasma after this had been stored with its normal content of platelets at −20 C for 24 hours gave a normal thrombin generation Recalcification time 2 min 15 sec

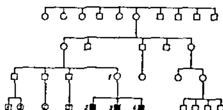
Curve G The patient s platelet poor plasma added to platelet rich plasma from pt no 10 fam 4 (Christmas disease) was unable to normalise the thrombin generation of the latter though considerable improvement was obtained Recalcification time 5 min 30 sec

Diagnosis Deficiency of the antihaemophilic factor plus freezing/serum factor

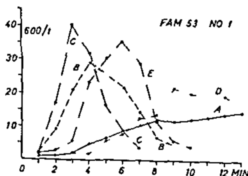
Comments

The result of experiment G suggests slight deficiency of the Christmas factor in addition to the AHF deficiency

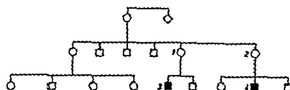
Family 53



1 Born 191? The patient is liable to subcutaneous haemorrhages which are the only signs of a bleeding tendency



Family 52

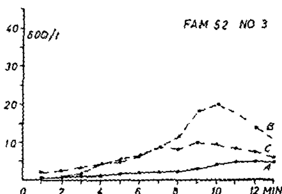


3 Born 1921 Propositus A bleeding tendency was manifest from the age of 4 years In infancy he most often had prolonged oozing gingival haemorrhages but not epistaxis From school age there were increasingly frequent painful haemarthroses gradually comprising all the large joints chiefly the ankles and knees where they have left permanent changes in the form of limited mobility The patient has had numerous intramuscular haemorrhages At the age of 25 dental extraction was followed by bleeding lasting 10 days At 26 he had haematuria At 36 he was admitted to hospital owing to anaemia and fatigue During the stay in hospital the patient was found to have pneumonia and a pulmonary abscess The patient died after several minor haemophtyses Post mortem diagnosis Haemophilia abscess of the right inferior pulmonary lobe pulmonary oedema grave anaemia chronic bilateral calculous pyelonephritis adhesive fibrosis of both pleurae mild to moderate atherosclerosis of the coronary arteries and aorta mild to moderate dilatation and hypertrophy of the left ventricle of the heart acute hyperplasia of the spleen The patient was practically unfit for work on account of his disease which had not grown milder with increasing years

Blood analyses 1946 (The Alborg Amtssygehus Med Dept) Prothrombin index 89 % Thrombocytes 420 000 Clotting time 72 min Bleeding time 2 min

1957 (The Alborg Amtssygehus Med Dept) Prothrombin concentration 90 % Thrombocytes 195 000 Fibrinogen 230 mg % Clotting time 34 min Bleeding time 2½ min

Present investigations Quick s prothrombin time 17 17 17 sec (control 17 sec) Thrombocytes 284 000 per µl of plasma Recalcification time in dilute plasma 10 min 30 sec The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma Some rise of the thrombin concentration Recalcification time 4 min 10 sec

Curve C Addition of 0 2 ml of normal serum caused no rise of the thrombin concentration *Recalcification time* 4 min 38 sec

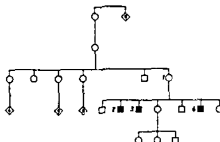
Curve D Addition of heated readsorbed normal serum A slow rise of the thrombin concentration Recalcification time 7 min 40 sec

Curve E Simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 2 ml of heated readsorbed serum The thrombin generation normal Recalcification time 3 min 5 sec

Curve E Addition of 0 2 ml of platelet suspension Some rise of the thrombin concentra tion after a lag period of 6 min Recalcification time 7 min 30 sec
Curve F Addition of 0 . ml of heated readsorbed serum The thrombin generation poor Recalcification time 7 min 30 sec

Diagnosis Christmas factor deficiency (Christmas disease)

Family 54

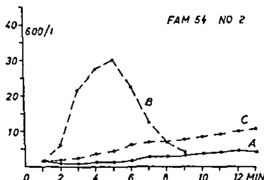


2 Born 1925 Propositus A bleeding tendency was observed from the first year of life At first this manifested itself chiefly by bleedings from the gums and nose as well as spontaneous haemorrhages into the skin Since the age of 3–4 years he has had increasingly frequent haemorrhages into the large joints especially the knees the mobility of which has gradually become permanently limited There is atrophy of the muscles of both legs The patient has had several episodes of haematuria accompanied by lumbar pain There have been numerous intramuscular bleedings The disease seems not to have decreased in severity in the course of years The patient is disabled and receives disable ment benefit

Blood analyses 1939 (The Thisted Sygehus) Howell Gram 15 min Blood platelets 240 000 Bleeding time 2 min

1948 (The Thisted Sygehus) Clotting time 7½–15 min Blood platelets 420 000

Present investigations Quick s prothrombin time 21 20 18 sec (control 20 sec) Thrombocytes 186 000 per µl of plasma Recalcification time in dilute plasma 11 min 30 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation nor mal Recalcification time 2 min 15 sec
Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcifica tion time 6 min

Diagnosis Defi iency of the antihaemophilic factor (classical haemophilia)

Blood analyses Quick s prothrombin time 19 19 20 sec (control 20 sec) Thrombocytes 99 000 per μl of plasma Recalcification time in dilute plasma 4 min 20 sec The thrombin generation test showed a very slowly rising thrombin concentration (Curve A)

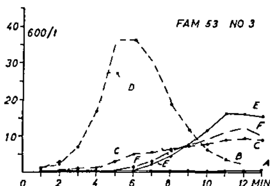
Curve B Addition of 0 2 ml of platelet suspension (141 000 platelets per μl of suspension) to the patient s blood normalised the thrombin generation Recalcification time 2 min
Curve C After storage of the patient s plasma with its normal content of platelets at −20 C for 3 months the thrombin generation was found to be perfectly normal Recalcification time 2 min 15 sec
Curve D Addition of 0 2 ml of fresh patient plasma to 1 ml of citrated plasma from the patient mentioned below (no 3) with manifest haemophilia gave only slight improvement of the patient s thrombin generation Recalcification time 5 min 15 sec
Curve E Addition of plasma stored at −20 C to citrated plasma from the patient mentioned below gave a normal thrombin generation with a steep rise and fall of the thrombin concentration Recalcification time 3 min 30 sec

Diagnosis Impaired thromboplastic activity presumably due to a reduced content of Christmas factor

3 Born 1935 Propositus A bleeding tendency was noticed from the age of 4 months manifesting itself by bleedings from the gums and nose as well as profuse haemorrhages into the skin The patient has had several episodes of melaena and one of haematemesis There has repeatedly been haematuria The patient has had two or three retrobulbar and subconjunctival haemorrhages causing protrusion of the eyeball and eversion of the eyelids During these episodes the patient had diplopia He has had several intramuscular haemorrhages in the right hand among others (claw hand) He has had numerous haemarthroses in the knees ankles elbows and wrists which have resulted in permanently limited mobility of the elbows knees and ankles The right forearm is about 10 cm shorter than the left The head of the radius is dislocated ulnarly The right hand presents 40 degrees ulnar flexion

Blood analyses 1937 (The Roskilde Amtssygehus) Howell Gram 1) 10 min 2) 28 min Thrombocytes 500 000 Bleeding time 3 min

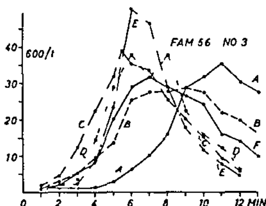
Present investigations Quick s prothrombin time 21 20 20 sec (control 20 sec) Thrombocytes 240 000 per μl of plasma Recalcification time in dilute plasma 19 min Clotting time for whole blood at 37 C 28 min The thrombin generation test showed no thrombin generation (Curve A)



Curve B No signs of an inhibitor Addition of 0 2 ml of normal citrated plasma effected a normal thrombin generation Recalcification time 3 min 20 sec
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 5 min
Curve D Addition of 0 2 ml of serum The thrombin generation normal Recalcification time 2 min 30 sec

Blood analyses (Andreassen) Clotting time 18–22½ min Blood platelets 369 000 Fibrinogen in plasma 0 32 %

Present investigations Prothrombin time 19 18 19 sec (control 19 sec) Recalcification time in dilute plasma 6 min 20 sec The thrombin generation test showed very considerable thrombin generation after a lag period of 5–6 min The inactivation proceeded very slowly (Curve A)



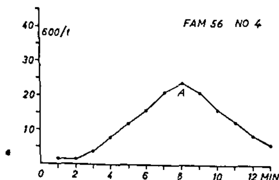
Addition of plasma either from a patient with Christmas disease (fam 23 no 3) or from one lacking the AHF (fam 76 no 1) normalised the thrombin generation (Curves B and C) The recalcification times were 3 min 45 sec and 2 min 30 sec

Addition of 0 2 ml of heated readsorbed serum or 0 4 ml of platelet suspension likewise gave a normal thrombin generation (Curves D and E) The recalcification times were 4 min and 4 min 15 sec

Diagnosis Deficiency of the Hageman factor

About 2 years after the above investigation the patient s blood was examined again Quick s prothrombin time 16 17 17 sec (control 17 sec) Thrombocytes 448 000 per µl of plasma The thrombin generation test showed a perfectly normal thrombin generation (Curve F) Recalcification time 3 min 20 sec

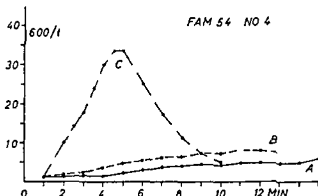
4 Born 1934 The patient displayed a bleeding tendency from the age of 2 years when a minor sore of the lip was followed by prolonged copious bleeding After this there were often prolonged haemorrhages from the nose and mouth Haemarthroses were never observed Since the age of 14 the patient has noticed no abnormal bleeding phenomena



4 Born 1934 From the age of 6 months there were bleedings from the nose and mouth Dentition was attended by bleedings From the age of one year there were re current bleedings into the skin after minor injuries Since the age of 4 years the patient has had haemarthroses comprising primarily the knees the left elbow and the right hip The mobility of the right knee is limited and there is atrophy of the entire right leg The mobility of the left elbow is slightly limited He has had several episodes of haematuria with associated lumbar pain but never haematemesis nor melaena

Blood analyses 1956 (The Thisted Sygehus) Thrombocytes 296 000 Clotting time for whole blood over 2 hours Recalcification time 16 min Prothrombin time 24 sec (control 24 sec)

Present investigations Quick s prothrombin time 18 18 17 sec (control 18 sec) Thrombocytes 172 000 per µl of plasma Recalcification time in dilute plasma 9 min. The thrombin generation showed negligeable thrombin generation (Curve A)

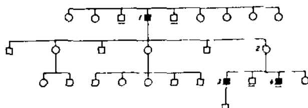


Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recal cification time 5 min

Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation nor mal Recalcification time 1 min 40 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 56

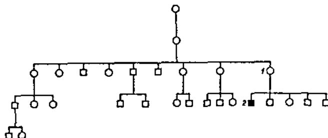


3 Born 1929 Propositus A bleeding tendency was first observed when the patient was 7 years old manifesting itself by prolonged oozing haemorrhages on second dentition He has had several episodes of prolonged epistaxis one of which was so severe as to necessitate admission to hospital From the age of 6–7 years the patient had haemorrhages into both knees and one elbow These have left no permanent changes He has had numerous profuse subcutaneous haemorrhages often appearing spontaneously Since the age of 14 the patient has experienced no abnormal bleeding phenomena apart from occasional bruises after violent injuries The patient is a keen football player and feels no troubles He is a pianist and is fully fit

Curve B Addition of 0 2 ml of normal serum The thrombin generation improved though still poor Recalcification time 6 min
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation nor mal Recalcification time 2 min 50 sec
Curve D Addition of 0 2 ml of readsorbed serum The thrombin generation normal Recalcification time 3 min

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 58

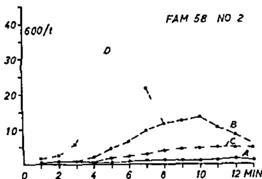


The family immigrated about 1850 At that time no haemophilic members were known

2 Born 1935 Propositus A bleeding tendency manifested itself from the age of about one year as oozing haemorrhages from the gums and nose often so severe as to necessitate admission to hospital and treatment by blood transfusion In 1937 he was operated on for incarcerated umbilical hernia (The Odense Amtssygehus) There was prolonged profuse secondary bleeding which however stopped spontaneously From the age of 4–5 years he has had haemarthroses comprising chiefly the elbows and knees where they have caused limited mobility He has had several episodes of haematuria haematemesis epistaxis and ecchymoses The patient is greatly disabled is unfit for any work, and receives disablement benefit

Blood analyses 1937 (The Odense Amtssygehus) Bleeding time 5 min 30 sec 1956 (The Centralsygehuset Svendborg Med Dept) Clotting time 7 min 30 sec Bleeding time 3 min Bexelius No petechiae Prothrombin time 28 sec (control 22 sec) Serum proteins 6 2 % X ray of the knees and elbows revealed arthrosis

Present investigations Quick s prothrombin time 20 19 19 sec (control 19 sec) Thrombocytes 335 000 per µl of plasma Recalcification time in dilute plasma 15 min The thrombin generation test showed no thrombin generation (Curve A)

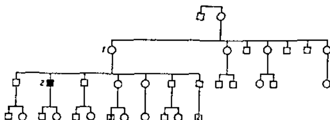


Blood analyses (Andreassen) Clotting time 11–16 min Blood platelets 437 000 Fibrinogen 0 39 % Bleeding time 4 min

Present investigations Quick's prothrombin time 20 20 20 sec (control 17 sec) Thrombocytes 235 000 per μl of plasma Recalcification time in dilute plasma 4 min The thrombin generation test showed a perfectly normal thrombin generation and inactivation (Curve A)

Diagnosis No detectable clotting defect

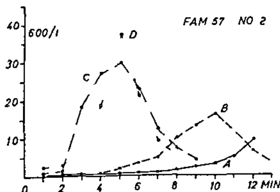
Family 57

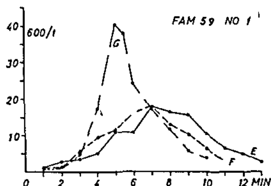


2 Born 1913 Propositus A bleeding tendency was noticed from the age of 2–3 years manifesting itself at first chiefly by subcutaneous haematomas on the body and later by bleedings from the gums and nose A few of these were life threatening and were treated by blood transfusions with good effect From school age he has had haemarthroses specially in the knees and hips Some of these were so severe as to keep the patient in bed for long periods The mobility of the left hip joint is slightly limited X ray of the right knee showed decreased density of the medial condyle of the femur There are no other permanent changes of the joints The patient has had several profuse intramuscular bleedings At the age of 42 he had a single episode of haematuria but there have been no other visceral bleedings After the age of 16 the abnormal bleeding phenomena began to decrease in frequency and intensity now rarely troubling the patient He has done gymnastics over a period of 10 years with difficult agility exercises without these having caused him any trouble The patient is an attendant and also keeps a shop

Blood analyses 1937 (The Aarhus Amtssygehus) Howell Gram 35 min Blood platelets 772 000 Bleeding time 1 min 30 sec Plasma fibrinogen 0 51 % 1956 (The Aarhus Kommunehospital) Clotting time 9 min Bleeding time 4 min 30 sec Capillary resistance No petechiae

Present investigations Quick's prothrombin time 18 18 18 sec (control 16 sce) Thrombocytes 331 000 per μl of plasma Recalcification time in dilute plasma 7 min 45 sec The thrombin generation test showed negligeable thrombin generation (Curve A)





Curve E. Addition of 0 2 ml of heated readsorbed serum as well as 0 2 ml of adsorbed bovine plasma The thrombin generation normal though the rise proceeded at a somewhat slower rate than in the pre eding experiment Recalcification time 2 min 42 sec

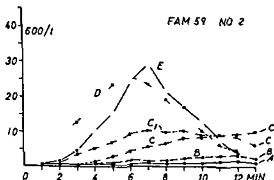
Curve F Addition of 0 2 ml of adsorbed bovine plasma as well as 0 2 ml of platelet suspension (145 000 per µl of suspension) The thrombin generation normal Recal cification time 2 min 37 sec

Curve G After storage of the patient s plasma at −20 C for 7 months addition of 0 2 ml of adsorbed bovine plasma alone effected a normal thrombin generation Recal cification time 3 min 15 sec The platelet content was 7000 per µl of plasma.

Diagnosis Deficiency of the antihaemophilic factor plus freezing/serum factor

2 Born 1906 From early infancy even the slightest injury gave rise to abnormal bleeding The patient has had many haemarthroses These have left permanent joint deformities a 35 degrees extension defect of the left elbow the right knee is fixed in extension The patient has had a single episode of haematuria but never melaena nor epistaxis The disease runs a definitely intermittent course It has grown milder with increasing years The patient is now handicapped only little by his disease The patient owns a wood working factory and is able to support himself and a family

Blood analyses Quick s prothrombin time 19 18 18 sec (control 18 sec) Thrombo cytes 306 000 per µl of plasma Recalcification time in dilute plasma 12 min 45 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 ? ml of serum The thrombin generation poor Recalcifi ation time 6 min 25 sec

Curves C and C Addition of 0 ? ml or 0 4 ml of adsorbed bovine plasma The thrombin

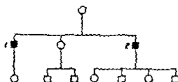
Curve B Addition of 0 2 ml of bovine plasma gave a slight delayed rise of the thrombin concentration Recalcification time 4 min 45 sec

Curve C Addition of 0 2 ml of serum A very low rise of the thrombin concentration Recalcification time 6 min 30 sec

Curve D Addition of 0 2 ml of adsorbed bovine plasma as well as 0 2 ml of normal serum The thrombin generation normal Recalcification time 3 min

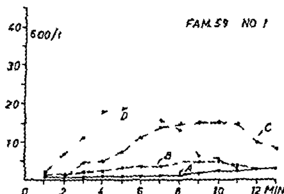
Diagnosis Deficiency of the antihaemophilic factor plus Christmas factor

Family 59



1 Born 1892 A bleeding tendency has been manifest since early infancy The patient is liable to subcutaneous haematomas both following injuries and spontaneous Even minor cuts are followed by prolonged bleedings Large haematomas develop after intra muscular injections The patient has had numerous haemarthroses which have left perma nent changes in some of the joints Both elbow joints are fixed at 130 degrees They can be moved only few degrees The ankles are fixed in 90 degrees flexion Both knee joints are stiff in extension The patient has had several episodes of painful haematuria but never haematemesis nor melaena He has never had a tooth extracted but is liable to gingival haemorrhages There have been several episodes of prolonged epistaxis After traumatic amputation at the age of 62 of the distal and middle phalanges of the left second and fifth fingers the patient had to be given three blood transfusions with excellent effect The patient is greatly disabled The disease seems not to have grown milder with increasing years but the patient nevertheless manages as a businessman

Blood analyses Quick s prothrombin time 17 17 17 sec (control 18 sec) Thrombo cytes 245 000 per μl of plasma Recalcification time in dilute plasma 16 min 50 sec The thrombin generation test showed negligeable thrombin generation (Curve A)

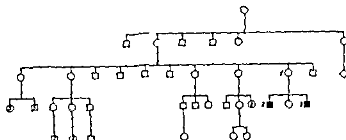


Curve B Addition of 0 2 ml of normal serum No essential improvement of the thrombin generation Recalcification time 3 min 41 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma A slow rise of the thrombin concentration which never attained particularly high values Recalcification time 3 min 36 sec

Curve D Addition of 0 2 ml of adsorbed bovine plasma as well as 0 2 ml of normal serum The thrombin generation normal Recalcification time 1 min 43 sec

Family 62

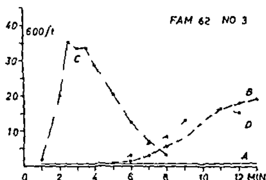


3 Born 1939 Propositus 6 months old the patient developed a large haematoma on the arm after an injury He is liable to large painful bruises Minor sores cause prolonged bleeding He has had many episodes of prolonged epistaxis and several episodes of haematuria but never haematemesis nor melaena From the age of 10 there were frequent severe bleedings into the knees elbows and ankles which have left permanent changes Both elbows present an extension defect of 30 degrees The left knee which is deformed can be moved from 180 til 170 degrees The patient is greatly affected by his disease He was unable to attend school He lives with his parents and earns a little money by making leather goods

Blood analyses 1939 (The Herning Sygehus) Howell-Gram 38 min Blood platelets 458 000 Bleeding time 4 min 30 sec Bexelius No petechiae Blood group A

1955 (The Tarm Sygehus) Clotting time 38 min Thrombocytes 458 000

Present investigations Quick s prothrombin time 19 19 20 sec (control 19 sec) Thrombocytes 589 000 per µl of plasma Recalcification time in dilute plasma 17 min The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma Some improvement of the thrombin generation after a lag period of 6–7 min Recalcification time 7 min

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 1 min 30 sec

Curve D Addition of 0 2 ml of heated readsorbed serum Slight improvement of the thrombin generation Recalcification time 6 min

Curve F shows the course of the thrombin concentration in the patients plasma after this had been stored with its normal content of platelets at –20 C for 15 days The thrombin generation was improved but not normal Recalcification time 6 min

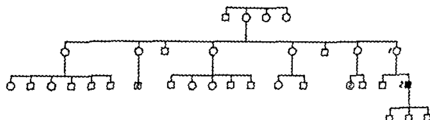
Curve F Addition of 0 5 ml of frozen platelet suspension (205 000 per µl) to the patients plasma effected fast formation and inactivation of thrombin Recalcification time 3 min

generation only slightly improved The recalcification times were 2 min 45 sec and 4 min 30 sec respectively

Curves D and E Addition of 0 2 ml of adsorbed bovine plasma to the patient's plasma after this had been stored at −20 C for 6 day The thrombin generation normal no matter whether the patient's plasma was platelet rich or platelet poor The recalcification times were 2 min 20 sec and 3 min respectively

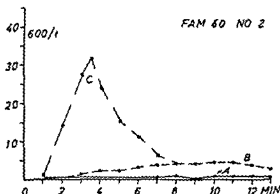
Diagnosis Deficiency of the antihaemophilic factor plus freezing/serum factor

Family 60



2 Born 1918 A bleeding tendency was detected when the patient at the age of about one year had a prolonged oozing bleeding from a small sore in the tongue 3 years old he had a life threatening haemorrhage after extraction of two teeth At the age of 34 he had a single episode of prolonged epistaxis From the age of 8–9 he had numerous painful bleedings into the large joints chiefly the ankles knees and hips There was transitorily limited mobility of the knee joints but permanent changes have occurred in none of the joints The patient has never had haematemesis nor melaena In adolescence he had a few episodes of haematuria The disease has grown considerably milder since the age of 15 now causing the patient little trouble The patient is a watch maker and able to live by his work He does not receive disablement benefit

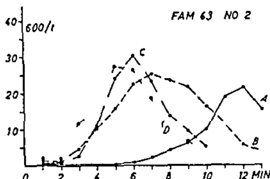
Blood analyses Quick's prothrombin time 19 18 18 sec (control 19 sec) Thrombocytes 334 000 per μl of plasma Recalcification time in dilute plasma 12 min 45 sec The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 4 min 45 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min

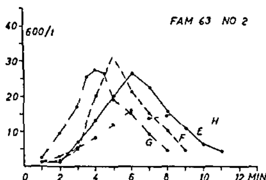
Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 30 sec

Curve C Addition of 0 2 ml of heated readsorbed serum The thrombin generation normal Recalcification time 3 min 25 sec

Curve D shows the course of the thrombin concentration in the patient s platelet poor plasma (4000 platelets per μl) after addition of 1 ml of a fresh platelet suspension containing 421 000 platelets per μl These platelets originated from his own plasma and had been washed three times in physiological saline The thrombin generation was normal Recalcification time 2 min 45 sec



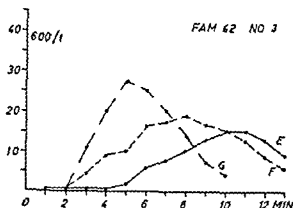
Curve E To investigate whether an inhibitor was present in the wash water of the platelets 1 ml of this was added to 1 ml of control plasma Curve E illustrates the thrombin generation in the plasma before addition of the wash water and curve F that after addition of the wash water The recalcification times were 3 min. and . min 45 sec respectively Inhibition of the thrombin generation was not demonstrable

Curve G The thrombin generation in the patient s plasma after this had been stored with its normal content of platelets at −20 C for 48 hours Recalcification time 1 min 40 sec

Curve H shows the result of a similar experiment with the patient s platelet poor plasma (18 000 platelets per μl of plasma) The course of the curve must be characterised as normal Recalcification time 2 min 45 sec

The patient s plasma normalised the thrombin generation in plasma lacking the Christmas factor and in AHF deficient plasma

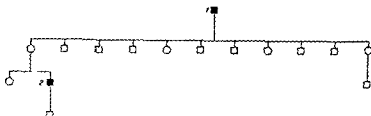
Diagnosis Deficiency of the Hageman factor



Curve G Addition of 0 2 ml of serum from a patient with AHF deficiency (fam 67) The thrombin generation normal Recalcification time 2 min 40 sec

Diagnosis Christmas factor deficiency (Christmas disease)

Family 63



The family is known several generations back but cases of haemophilia are not known for certain to have been present in the elder generations Several family members live abroad

2 Born 1892 Propositus A bleeding tendency was first observed at the age of 10 when incision of an abscess was followed by prolonged oozing haemorrhage The wound was sutured twice before the bleeding stopped Later the patient had frequent episodes of prolonged often spontaneous epistaxis but these ceased after the age of 30 When young he was also liable to large subcutaneous haematomas In 1941 the patient had haematuria with attending discharge of concretions In 1941 he also had a severe attack of haem atemesis without preceding dyspeptic complaints He was admitted to hospital where no other cause of the patient s haematemesis was detectable than the haemophilia Transfusion was necessary to stop the bleeding There have been 7–8 episodes of melaena At the age of 64 the patient had a prolonged bleeding after dental extraction which necessitated transfusion

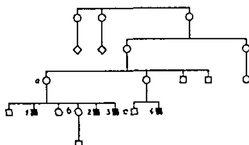
The patient is little handicapped by his disease He manages his work as a debt collector riding uphill and downhill on bicycle without ever taking account of his disease

Bloodanalyses 1941 *(The Hillerod Sygehus) Howell Gram 8 min* Thrombocytes 400 000 Bleeding time 4 min

1942 *(Andreassen)* Clotting time 13¾–19 min clot loose Bleeding time 4½ min Thrombocytes 343 000

Present investigations Quick s prothrombin time 15 15 15 sec (control 15 sec) Thrombocytes 448 000 per µl of plasma *Recalcification time in dilute plasma 6 min* 45 sec The thrombin generation test showed after a lag period of 6 min a slow rise of the thrombin concentration which nevertheless attained high values (Curve A)

Family 66



1 Born 1933 When the patient was about one year old a bleeding tendency manifested itself by swelling of the knees after injuries One year old he fractured his right knee Later he had many episodes of epistaxis which twice necessitated blood transfusion He died 2 years old of pneumonia

Blood analyses (The Rigshospitalet Paediatric Dept) 1935 Thrombocytes 354 000 Rumpel Leed No petechiae Bleeding time 5–6 min Howell Gram 15 min

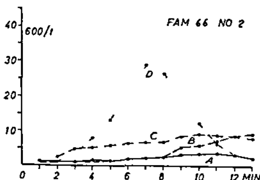
Diagnosis Haemophilia (not classified)

2 Born 1939 Propositus There have been abnormal bleeding phenomena since the age of one year Ecchymoses and frequent bleedings into the elbows shoulders hips and knees There is limited mobility of the left knee which can be moved from 120 to 80 degrees The left elbow is fixed at about 130 degrees There is pronounced atrophy of the extremital muscles The patient has had several episodes of melaena and haematemesis as well as one of haematuria He has bled a few times from the gums and nose The patient is under public care for mental defectives

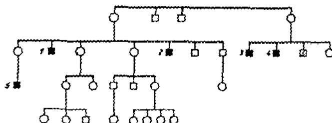
Blood analyses 1957 (The Centralsygehuset Slagelse Med Dept) Thrombocytes 44 000 per µl of plasma Bleeding time 3 min 10 sec Clotting time 10 min Prothrombin proconvertin (Owren) 66 %

Present investigations (1956) Quick s prothrombin time 17 16 17 sec (control 17 sec) Thrombocytes 283 000 per µl of plasma Recalcification time in dilute plasma 14 min The thrombin generation test showed negligeable thrombin generation (Curve A)

1957 Quick s prothrombin time 18 18 19 sec (control 19 sec) Prothrombin proconvertin (Owren) 100 % Thrombocytes 76 000 per µl of plasma Recalcification time in dilute plasma 9 min 45 sec The thrombin generation test showed negligeable thrombin generation (Curve B)



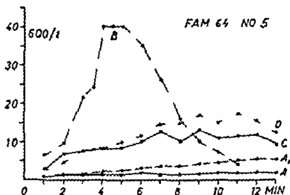
Family 64



5 Born 1912 Propositus The patient has had many haemarthroses since the age of 2 years Nearly all the joints have been involved and several of these have become permanently changed The right elbow can be moved from 45 to 160 degrees The olecranon is dislocated backwards The muscles of the upper arm are highly atrophic The left elbow presents 30 degrees extension defect The right knee is nearly fixed in extension The left knee can be bent 30 degrees The patient has had several episodes of haematuria with no attending pain but never haematemesis nor melaena He has had a few episodes of epistaxis There have been many subcutaneous haematomas He feels that the disease has grown milder with increasing years The patient who is greatly disabled is a man of independent means He keeps poultry to supplement his income He has received about 25 transfusions

Blood analyses 1942 (*Andreassen*) Clotting time (Burker) 31½–42 min

Present investigations Quick s prothrombin time 19 18 19 sec (control 18 sec) Thrombocytes 252 000 per µl of plasma Recalcification time in dilute plasma 20 min The thrombin generation test showed negligeable thrombin generation (Curve A) Curve A_1 illustrates the thrombin generation in a blood sample withdrawn about 1½ years after the above The recalcification time was then 12 min 10 sec The thrombin generation was still very poor

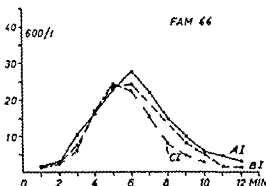


Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min

Curve C Addition of 0 2 ml of normal serum Little improvement of the thrombin generation Recalcification time 2 min 30 sec

Curve D Addition of 0 2 ml of readsorbed serum gave some further improvement of the thrombin generation though not to the normal level Recalcification time 2 min 20 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

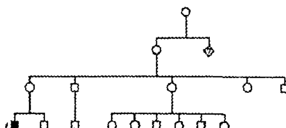


Blood analyses Thrombocytes 333 000 per µl of plasma Recalcification time in dilute plasma 3 min The thrombin generation test showed a normal thrombin generation (Curve B)

c Born 1936 A brother of pt no 4 He has never displayed signs of a haemorrhagic diathesis

Blood analyses Quick s prothrombin time 18 18 18 sec (control 18 sec) Thrombocytes 473 000 per µl of plasma Recalcification time in dilute plasma 3 min 25 sec The thrombin generation test showed a normal thrombin generation (Curve C_1)

Family 67



The propositus is the only haemophiliac known in the family

1 Born 1945 Propositus At birth the patient had a large cephalic haematoma which persisted for one year 2 years old he had blood accumulations in the right and the left ankles after an injury Since there have been frequent bleedings into the knees elbows shoulders hips wrists and finger joints but these have left no permanent changes On second dentition there were prolonged oozing haemorrhages He has never had epistaxis haematemesis melaena nor haematuria The patient bruises easily His parents think that the disease has grown somewhat milder with increasing years The patient has never been given blood transfusions

Blood analys s Qu ck s prothrombin time 18 17 18 sec (control 18 sec) Thrombocytes 92 000 per µl of plasma Recalcification time in dilute plasma 6 min 15 sec The thrombin generation test showed a very poor thrombin generation (Curve A)

Curve C Addition of 0 2 ml of normal serum No essential improvement of the thrombin generation Recalcification time 4 min

Curve D Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 30 sec

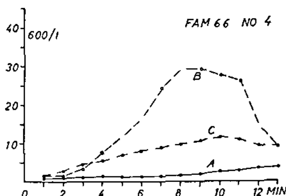
Freezing of the plasma addition of platelet suspension or readsorbed serum gave no essential improvement of the thrombin generation Addition of 0 2 ml of adsorbed bovine plasma as well as 0 2 ml of heated readsorbed serum effected no better thrombin generation than addition of bovine plasma alone

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

3 Born 1944 From the age of one year the patient had prolonged bleedings into the skin He has had several episodes of haematemesis and melaena and very often epistaxis He has been given several transfusions There have often been bleedings into the elbows knees and ankles These have left permanent joint deformities There is 10 degrees extension defect of both elbows The contours of the knee joints are blurred and the mobility limited In 1956 the patient was admitted to hospital with a feeling of heaviness in the stomach followed by haematemesis He died 24 hours after admission

4 Born 1946 A bleeding tendency was observed from the patient was about one year old manifesting itself by frequent bleedings of unknown cause into the tissues as well as frequent haemarthroses especially into the knees There are permanent changes in the left knee which presents 50 degrees extension defect He has never had haematemesis melaena nor haematuria

Blood analyses Quick s prothrombin time 18 19 18 sec (control 18 sec) Thrombocytes 503 000 per µl of plasma Recalcification time in dilute plasma 18 min 15 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 30 sec

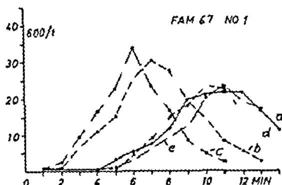
Curve C Addition of 0 2 ml of serum Little improvement of the thrombin generation Recalcification time 3 min 30 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

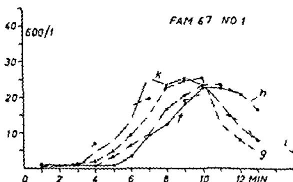
a Born 1910 The patient has never displayed signs of a haemorrhagic diathesis

Blood analyses Quick s prothrombin time 18 18 18 sec (control 18 sec) Thrombocytes 191 000 per µl of plasma Recalcification time in dilute plasma 3 min The thrombin generation test showed a normal thrombin generation (Curve A₁)

b Born 1936 A sister of pt no 2 She has never displayed signs of a haemorrhagic diathesis She has a son who likewise has displayed no signs of a haemorrhagic diathesis

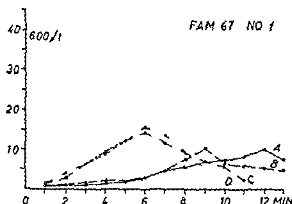


Curve a illustrates the thrombin generation in the patient's plasma After a lag period of just over 4 min the thrombin concentration rose slowly to reach high values
Curve b Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 39 sec
Curve c Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 2 min 27 sec
Curve d Addition of 0 2 ml of heated readsorbed serum gave no improvement of the thrombin generation Recalcification time 6 min 15 sec
Curve e The patient's own serum was unable to normalise the thrombin generation Recalcification time 6 min 30 sec



Curve f Addition of 0 5 ml of fresh platelet suspension (300 000 per µl) No improvement of the thrombin generation Recalcification time 6 min 30 sec
Curve g Frozen platelet suspension (205 000 per µl) gave som acceleration of the thrombin generation Recalcification time 4 min 40 sec
Curve h Addition of 0 5 ml of plasma from a patient with AHF deficiency (fam 31 no 6) to 0 5 ml of this patient's plasma did not result in a normal thrombin generation Recalcification time 5 min 35 sec
Curve i On adding 0 5 ml of the patient's plasma to 0 5 ml of plasma from a patient with Christmas disease the thrombin generation became normal (fam 62 no 3) Recalcification time 3 min 15 sec
Curve k In a similar experiment the patient's plasma was mixed with plasma from a patient with the Hageman trait (fam 112 no 1) The thrombin generation was normal in the mixture Recalcification time 3 min 40 sec
The patient's plasma could not normalise the thrombin generation in AHF-deficient plasma (fam 2 no 11)

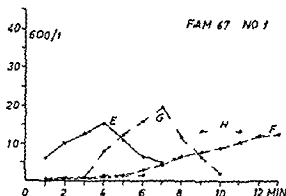
Diagnosis Slight AHF deficiency



Curve B Addition of 0 2 ml of frozen platelet suspension containing 135 000 platelets per µl of plasma No essential improvement of the thrombin generation Recalcification time 6 min 35 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma A steep rise and fall of the thrombin concentration which did not reach high values Recalcification time 2 min 30 sec

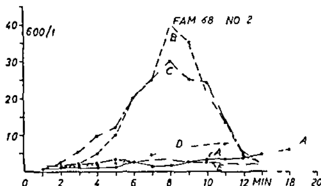
Curve D Similar conditions were found after addition of 0 2 ml of normal serum Recalcification time 2 min 20 sec



Curve E Simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 2 ml of normal serum to 1 ml of the patient s plasma gave no further improvement of the thrombin generation

The results of these experiments suggested PTA deficiency However addition of 0 2 ml of the patient s fresh plasma to plasma from a patient with classical haemophilia (fam 66 no 2) gave little improvement of the thrombin generation (curve F) Recalcification time 7 min Addition of 0 2 ml of the patient s plasma to plasma from a patient with Christmas disease (fam 4 no 10) (Curve G) effected a fairly steep rise and fall of this patient s thrombin concentration after a lag period of 3 min Recalcification time 3 min 45 sec Storage of the patient s platelet-containing plasma at −20 C for 17 days gave no essential improvement of the thrombin generation (Curve H) Recalcification time 6 min

Owing to the thrombopenia the investigation was repeated 6 months later Quick s prothrombin time 18 18 18 sec (control 20 sec) Thrombocytes 788 000 per µl of plasma Recalcification time in dilute plasma 5 min 15 sec



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal The recalcification time was somewhat prolonged however 5 min 45 sec

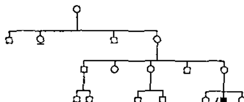
Curve C By adding 0 4 ml of adsorbed bovine plasma to the patient s plasma the thrombin generation was still normal The recalcification time was now 3 min 5 sec

Curve D Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 8 min

Curve E Addition of 0 2 ml of readsorbed serum The thrombin generation poor Recalcification time 5 min

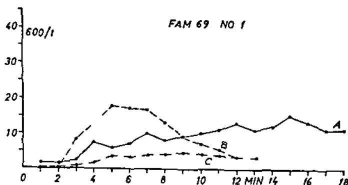
Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 69

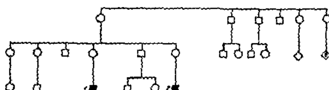


The propositus is the only haemophiliac known in the family

I Born 1950 Propositus The patient has bruised easily since he was about one year old He once bled for 3 days from the lip after a minor injury One year old he had a



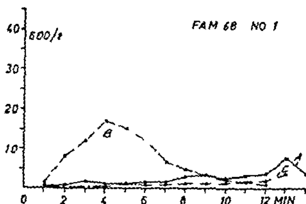
Family 68



1 Born 1938 Propositus A bleeding tendency manifested itself from the patient was *one year old as subcutaneous extravasations* Since the age of 2 years he has had bleedin s into the knees ankles and wrists Such have caused limited mobility of the knees and ankles He has had episodes of haematuria and prolonged epistaxis as well as a single episode of intra abdominal haemorrhage but never haematemesis nor melaena

Blood analyses (The Frederiksberg Hospital Dept B) Thrombocytes 600 000 Clotting time 7 min (control 6 min) Bleeding time 10 min Capillary resistance No petechiae Prothrombin 90 %

Present investigations Quick s prothrombin time 24 24 24 sec (control 22 sec) Recalcification time in dilute plasma 14 min The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 1 min 30 sec

Curve C Addition of 0 2 ml of normal serum No improvement of the thrombin generation Recalcification time 6 min

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

2 Born 1940 In early infancy the patient had a subcutaneous haematoma on the buttocks At the age of 7 he bled for a long time from a sore of the hand Several transfusions were required to obtain haemostasis From school age there have been frequent *haemarthroses* specially in the knees and ankles At the age of 14 he was admitted to hospital twice with haematuria and once with intramuscular bleeding in one thigh He bruises easily but was otherwise troubled but little by his disease the past 3 years *After this interval he had a posttraumatic haemorrhage into the right ankle* The bleeding stopped after a fairly short while but was followed by a spontaneous haemorrhage into the left ankle The patient has never had haematemesis nor melaena He is not particularly liable to epistaxis *He has never had a tooth extracted*

Blood analyses Quick s prothrombin time 23 23 23 sec (control 23 sec) Thrombocytes 500 000 per µl Spontaneous clotting time at 37 C about 1½ hours Recalcification time in dilute plasma 15 min *The thrombin generation test showed negligeable thrombin generation* (Curve A)

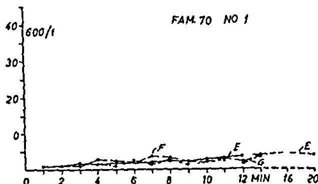
Blood analyses (The K H Dept III) Clotting time over 60 hours Bleeding time 13 min Thrombocytes 277 000 Bexelius No petechiae Fibrinogen 0 44 % –0 48 % Prothrombin proconvertin (Owren) 50 % –72 % Serum protein 6 %

Present investigations Prothrombin proconvertin (Owren) 100 % Prothrombin index 75 % Recalcification time in dilute plasma about 20 min The thrombin generation test showed no thrombin generation (Curve A)

Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 5 min

Curve C Addition of 0 2 ml of normal serum Slight improvement of the thrombin generation Recalcification time 4 min

Curve D Addition of 0 2 ml of normal plasma The thrombin generation poor Recalcification time 7 min



Curve E Addition of platelet suspension The thrombin generation poor Recalcification time 15 min

Curve F Addition of 0 4 ml of citrated plasma from a patient with Christmas disease (fam 4 no 7) The thrombin generation poor Recalcification time 5 min

Curve G Addition of 0 2 ml of citrated plasma from a patient with classical haemophilia (fam 69) The thrombin generation poor Recalcification time 7 min

The table fig 86 shows the thrombin fibrinogen reaction in the patient s plasma and in a control plasma A series of dilutions of thrombin solution were prepared To each tube was added 0 4 ml of patient and control plasma respectively The clotting time in the mixture was determined

Fig 86

Series of thrombin dilutions	1	/	¹/	¹/	¹/₁₆	¹/
Clotting time in pt pla ma	9	11	18	33	50	>4
Clotting time in control plasma	8	10	16½	31	57	>3

The thrombin fibrinogen reactions seemed to be uniform in the two experiments

The influence of the patient s plasma on the clotting time of normal plasma was investigated by adding increasing volumes of patient plasma (series of dilutions) to the control plasma and determining the recalcification time (see the table fig 87)

Fig 87

Control plasma (ml)	0 2	0 2	0 2	0 2	0 2
Patient plasma (ml)	0	0 025	0 05	0 1	0 2
Saline (ml)	0 2	0 175	0 15	0 1	0
Recalcification times	1 45	? 7	2 29	3 ‹6	3 40

bleeding into the right elbow joint and 5 years old epistaxis of 10 days duration He has had neither haematemesis melaena nor haematuria

Blood analyses 1955 (The Børnehospitalet Martinsvej) Thrombocytes 315 000 Capillary resistance No petechiae Prothrombin time (Plum and Larsen) normal Bleeding time 5–10–3 min

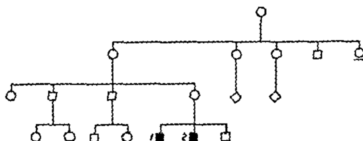
Present investigations Prothrombin proconvertin (Owren) 100 % Recalcification time in dilute plasma 4 min 30 sec The thrombin generation test showed a very slow thrombin generation (Curve A)

Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 1 min

Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 3 min 30 sec

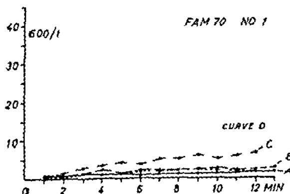
Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 70



We know of no other cases of haemophilia in the family

1 Born 1940 Propositus The patient has had frequent bleedings from the nose and gums since early infancy and since he began to crawl recurrent haemarthroses specially in the knees ankles shoulders and elbows The right knee is stiff after straightening of contracture There is limited mobility of the left knee joint and of both ankles The patient has had numerous intramuscular bleedings and bleedings into the skin There have been neither haematuria nor gastro intestinal haemorrhages The patient is greatly disabled and spends much of his time in a wheeled chair He thinks that the bleedings become worse after blood transfusions

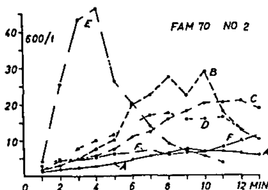


These thromboplastin concentrations were evidently too small to inactivate the inhibitor of the patient's plasma

Diagnosis Thromboplastin inhibitor

2 Born 1943 One year old the patient bled for a long time after having bitten his lip and later his tongue He has had many episodes of prolonged epistaxis and has always bruised easily He has had numerous bleedings into the knees and elbows where they caused slightly limited mobility There were also frequent bleedings into the right ankle and the left wrist The patient has had a few episodes of haematuria lasting up to 2 weeks but never haematemesis nor melaena He has never experienced gingival or intramuscular haemorrhages Several blood transfusions have been given which according to the patient were followed by prolonged bleedings

Blood analyses Quick's prothrombin time 18 19 18 sec (control 17 sec) Prothrombin proconvertin (Owren) 93 % Thrombocytes 472 000 per µl of plasma Recalcification times in dilute plasma 7 min The thrombin generation test showed very little thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 12 sec

Curve C Addition of 0 2 ml of serum The thrombin generation improved but not normal Recalcification time 3 min

Curve D Also addition of 0 2 ml of readsorbed serum effected some improvement of the thrombin generation without normalising it however Recalcification time 2 min 50 sec

Curve E No signs of an inhibitor The patient's plasma added to normal plasma did not alter the normal thrombin generation Recalcification time 1 min 30 sec

Curve F shows the thrombin generation in plasma stored at −20 C for 24 hours The thrombin generation unchanged poor Recalcification time 5 min 30 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

By adding increasing volumes of patient plasma the clotting time in the control plasma was seen to increase in length

Finally the influences of tissue thromboplastin on the recalcification times in the patient s plasma and in a control plasma were investigated A series of saline dilutions of Owren s thromboplastin were prepared (see the table fig 88)

Fig 88

Tube no	Series of saline dilutions of Owren thromboplastin Total volume 0 2 ml	CaCl M/40	Clotting time with patient plasma 0 ml	Clotting time with control plasma 0 2 ml
1	0 2 2^0	0 2	22	23
2	0 2 2^{-1}	0 2	21	24
3	0 2 2^{-2}	0 2	23	30
4	0 2 2^{-3}	0 2	29	43
5	0 2 2^{-4}	0 2	34	39
6	0 2 2^{-5}	0 2	40	47
7	0 2 2^{-6}	0 2	50	57
8	0 2 2^{-7}	0 2	1 6	1 6
9	0 2 2^{-8}	0 2	1 19	1 21
10	0 2 2^{-9}	0 2	1 40	1 44
11	0 2 2^{-10}	0 2	2 12	1 35
12	0 2 2^{-11}	0 2	7 57	2 6
13	0 2 2^{-12}	0 2	9 12	2 10
14	0 2 2^{-13}	0 2	9 06	2 17
15	0 2 2^{-14}	0 2	12 30	2 42

To each tube were added 0 2 ml of patient plasma and 0 2 ml of M/40 CaCl and the clotting time was determined (diluted one stage prothrombin time) Corresponding experiments were made with the control plasma In fig 89 the relations between thromboplastin concentrations and clotting times are represented graphically At thromboplastin concentrations below 2^{-9} the clotting times in the patient s plasma increased abruptly in length

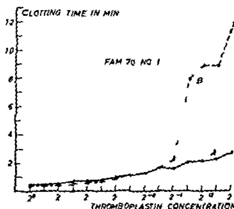


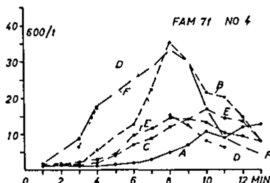
Fig 89

Relations between thromboplastin concentrations (abscissa) and clotting times (ordinate) in mixtures of Owren s thromboplastin and a control plasma (Curve A) as well as Owren s thromboplastin and patient plasma

4 Born 1949 From the age of 14 months the patient bruised easily and was liable to large haematomas after minor injuries Since the age of 18 months he has had frequent haemorrhages into the ankles knees elbows and wrists Gradually some irregularity has developed of the epiphyseal surfaces in the elbow joints The mobility is not limited He has never had melaena haematuria nor haematemesis

Blood analyses 1950 (The Rigshospitalet Paediatric Dept) Clotting time 8–11 min 35 sec Prothrombin time 23 sec (control 20 sec) Bleeding time 4½ min Capillary resistance (80 mm for 5 min) No petechiae Fibrinogen 0 41 %

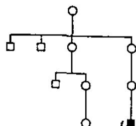
Present investigations Quick s prothrombin time 19 19 19 sec (control 18 sec) Thrombocytes 389 000 per µl of plasma Recalcification time in dilute plasma 8 min 9 sec The thrombin generation test showed a poor thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 4 min 10 sec
Curve C Addition of 0 2 ml of normal serum Some improvement of the thrombin generation which was poor Recalcification time 5 min 40 sec
Curve D Addition of 0 2 ml of readsorbed serum The thrombin generation normal Recalcification time 3 min 30 sec
Curve E Heated readsorbed serum gave only little improvement of the thrombin generation Recalcification time 5 min
Curve F Course of the thrombin generation in the patient s plasma after this had been stored at −20 C for 7 days The thrombin generation normal Recalcification time 3 min 15 sec

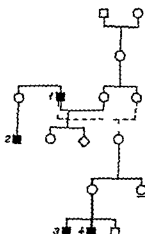
Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 72



There are no other known cases of haemophilia in the family

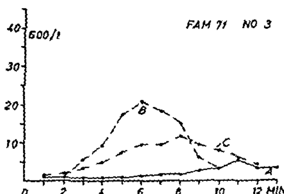
Family 71



3 Born 1947 Propositus The patient has bruised easily since birth and has been liable to haemarthroses since the age of one year chiefly in the elbows knees and ankles most often spontaneous The left elbow joint has become permanently changed with mild deformation of the articular surfaces The right knee lacks 45 degrees in full flexion At the age of 3 the patient bled for a long time from a sore of the lower lip Transfusion was necessary to stop the bleeding Minor cuts caused prolonged bleeding 4 years old the patient had prolonged oozing haemorrhage after dental extraction He has never had haematemesis melaena nor haematuria

Blood analyses 1953 (The Rigshospitalet Paediatric Dept) Clotting time 18–27 min *Bleeding time 3¼ min Prothrombin time 20 sec (control 17 sec) Thrombocytes* 245 000 1955 Clotting time 25–30 min Thrombocytes 386 000 Prothrombin time 18 sec (control 18 sec) Fibrinogen 0 38 % 0 58 % Bexelius No petechiae

Present investigations Quick s prothrombin time 16 16 16 sec (control 17 sec) Thrombocytes 640 000 *per µl Recalcification time in dilute plasma* 10 *min* The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 40 sec

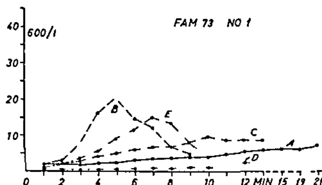
Curve C Addition of 0 2 ml of normal serum Little improvement of the thrombin generation Recalcification time 3 min 25 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

and oozing haemorrhage from a bite in the cheek 4 years old he had haematomas in various muscles as well as bleedings into the shoulders and ankles These have caused no permanent changes so far He has had neither haematemesis melaena haematuria nor epistaxis

Blood analyses 1953 (The Rigshospitalet Paediatric Dept) Thrombocytes 24? 000 Prothrombin time 21 sec (control 20 sec) Bleeding time 2¹ –over 12 min Capillary resistance (Gothlin) 0–3 petechiae Clotting time 45–120 min After transfusion 9 min

Present investigations Quick s prothrombin time 23 23 22 sec (control 20 sec) Recalcification time in dilute plasma 15 min The thrombin generation test showed negligible thrombin generation within 21 min (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 40 sec

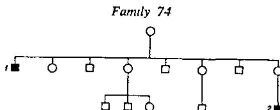
Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 4 min 30 sec

Curve D Addition of 0 2 ml of readsorbed normal serum The thrombin generation poor Recalcification time 13 min

Curve E Addition of 0 2 ml of adsorbed bovine plasma as well as 0 2 ml of serum effected no better thrombin generation than addition of adsorbed bovine plasma alone Recalcification time 2 min 35 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 74



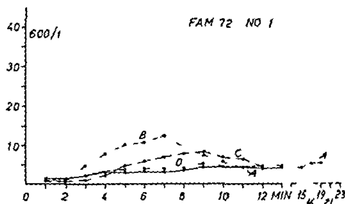
1 The patient died at the age of 2 years from epistaxis He is said to have had numerous haemarthroses

2 Born 1944 Propositus From early infancy the patient had frequent subcutaneous extravasations of blood He has had two episodes of prolonged bleeding in relation to secondary dentition Since the age of 2 years the patient has had repeated haemorrhages into the knees and ankles Arthrosis has developed in the right knee joint The left

1 Born 1945 Propositus A bleeding tendency was first noticed when the patient at the age of 6 months developed subcutaneous heamatomas Since the age of one year there were haemarthroses and intramuscular haemorrhages Nearly all the large joints have been subject to haemarthroses There is now limited mobility of the right knee and hip He has never had haematemesis melaena haematuria nor epistaxis The patient is greatly troubled by his disease He has been admitted to hospital several times to be given blood transfusions

Blood analyses 1955 (The Rigshospitalet Paediatric Dept) Prothrombin time 18 sec (control 18 sec) Thrombocytes 296 000 Fribrinogen 0 34 % Capillary resistance (Gothlin) No petechiae Clotting time 24–180 min After transfusion 7 min

Present investigations Prothrombin time 19 21 23 sec (control 21 sec) The thrombin generation test showed negligeable thrombin generation within 23 min (Curve A) Recalcification time in dilute plasma 5–12 min



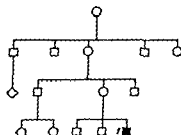
Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal *Recalcification time 2 min 42 sec*

Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 4 min 10 sec

Curve D Addition of 0 2 ml of readsorbed serum The thrombin generation poor Recalcification time 4 min

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

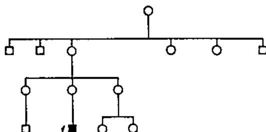
Family 73



No other cases of haemophilia are known in the family

1 Born 1951 Propositus From the age of 2 months the patient displayed signs of a haemorrhagic diathesis in the forms of subcutaneous haematomas and prolonged bleedings after minor injuries 2 years old he developed a large haematoma in the scrotum

Family 75

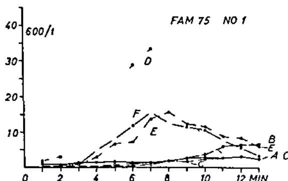


There are no known cases of haemophilia in the family beyond that described below

1 Born 1946 Propositus From the age of 5 months even the slightest hurts gave rise to ecchymoses 7 months old the patient had a prolonged oozing haemorrhage after a cut in the heel A blood transfusion was necessary The patient has had haemarthroses since the age of 11 months but there are no permanent changes as yet The patient has been admitted to hospital several times and has received many transfusions

Blood analyses (The Rigshospitalet Paediatric Dept) Prothrombin time 18 sec (control 21 sec) Thrombocytes 160 000–334 000 Clotting time 2½ hours Bleeding time 2 min Fibrinogen 0 45 % 1955 Clotting time before transfusion 60 → over 120 min After transfusion 6–8 min.

Present investigations Quick s prothrombin time 20 19 19 sec (control 19 sec) Thrombocytes 176 000 per µl of plasma Recalcification time in dilute plasma 17 min The thrombin generation test showed negligeable thrombin generation (Curve A)



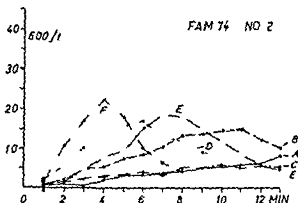
Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 8 min 50 sec
Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 8 min 15 sec
Curve D The thrombin generation was normal immediately after transfusion of 100 ml of fresh blood Recalcification time 3 min 45 sec
Curve E Simultaneous addition of 0 2 ml of serum and 0 2 ml of adsorbed bovine plasma gave a normal thrombin generation Recalcification time 4 min 30 sec
Curve F The same result was achieved by adding adsorbed bovine plasma to patient plasma stored at −20 C Recalcification time 4 min 30 sec

Diagnosis Deficiency of the antihaemophilic factor plus freezing/serum factor

knee presents a 20 degrees extension defect There has been no haematuria haematemesis melaena nor epistaxis The patient suffers from congenital syphilis and is mentally back ward He is under public care for mental defectives

Blood analyses 1954 (The Rigshospitalet Paediatric Dept) Prothrombin 69 %–90 % Clotting time 10–180 min Bleeding time 3 min –20 min Fibrinogen 0 52 % Thrombocytes 203 000–352 000 Capillary resistance No petechiae Serum protein 8 4 % Albumin 5 7 % Globulin 2 7 %

Present investigations Quick s prothrombin time 20 21 23 sec (control 19 sec) Prothrombin proconvertin (Owren) 120 % Thrombocytes 216 000 per µl of plasma *Recalcification time in dilute plasma 4 min 30 sec – 11 min 20 sec The thrombin generation* test showed a poor thrombin generation (Curve A)



Curve B Addition of 0 4 ml of adsorbed bovine plasma Some but very slow rise of the thrombin concentration Recalcification time 3 min 30 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 5 min 30 sec

Curve D Addition of 0 2 ml of adsorbed bovine plasma as well as *0 2 ml of normal serum The thrombin generation normal Recalcification time* 1 min 46 sec

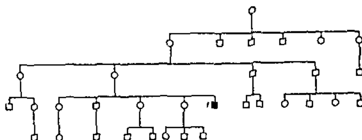
Curve E Addition of 0 2 ml of adsorbed bovine plasma as well as 0 2 ml of heated readsorbed serum The thrombin generation normal Recalcification time 3 min

Curve F Addition of 0 4 ml of adsorbed bovine plasma to plasma stored with its normal content of platelets at – 20 C for 24 hours gave a normal thrombin generation Recalcification time 1 min 45 sec

Diagnosis Deficiency of the antihaemophilic factor plus freezing/serum factor

This patient s fresh plasma could not be normalised by plasma lacking the AHF or by plasma lacking the Christmas factor whereas simultaneous addition of these two plasma samples normalised the thrombin generation The patient s blood must therefore be supposed to lack both the AHF and the Christmas factor *The latter defect may however* contrary to expectation *be substituted by a factor in heated readsorbed serum*

Family 77

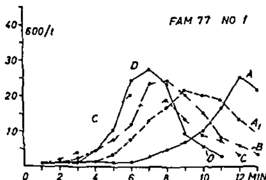


The propositus is the only known haemophilic member of the family

I Born 1935 Propositus One year old the patient bled for a long time from a cut in the tongue At the age of 3 years a copious amount of blood accumulated in the region of the left knee joint The patient has since had frequent haemorrhages into both knees wrists and the left elbow There is 20 degrees extension defect of the left elbow He has never had haematuria haematemesis nor melaena The patient has had several episodes of prolonged epistaxis Once at the age of 10 he had to have blood transfused to stop the bleeding He bruises rather easily and bleeds for a long time after minor lesions The patient often missed school on account of his disease which allegedly has abated within the last few years He has no permanent job and receives disablement benefit He is now preparing for a qualification as a telegraph operator and manages well

Blood analyses 1944 (The Rigshospitalet Paediatric Dept) Thrombocytes 249 000 Prothrombin time 34 sec (control 34 sec) Clotting time (Lundsten) 5 hours Clotting time according to Burker 19–25 min Fibrinogen 0 52 % Bexelius No petechiae

Present investigations Quick's prothrombin time 20 20 21 sec (control 20 sec) Thrombocytes 415 000 per μl of plasma Recalcification time in dilute plasma 8 min The thrombin generation showed a fairly fast rise of the thrombin concentration after a lag period of 6–7 min (Curve A) 18 months later the course of the thrombin generation was as illustrated by curve A_1 Recalcification time 5 min 40 sec

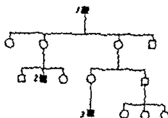


Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 45 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 3 min

Curve D Addition of 0 2 ml of heated readsorbed normal serum The thrombin generation normal Recalcification time 5 min

Family 76



The family comes from Germany

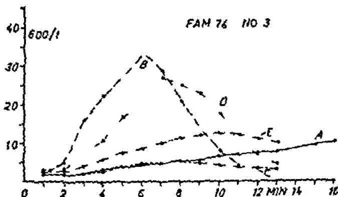
1 Born 1851 He was ostensibly a bleeder He died at home in 1914

2 Born 1915 The patient died 2 years old in a hospital in Germany *from haemorrhage from a cut in the right eyelid*

3 Born 1952 Propositus *From the age of 3 months the patient was liable to haematomas even after slight hurts 4 months old he had a fresh haemorrhage from the rectum One year old the patient was admitted to hospital with a large haematoma in the gluteal region 18 months old he bled from the frenulum labii sup He received three transfusions* with no unquestinable effect He has since been given several transfusions on account of subcutaneous haemorrhages *He has never had haemarthroses nor haematuria*

Blood analyses 1954 (The Rigshospitalet Paediatric Dept) Thrombocytes 32? 000 Clotting time 60–90 min *Prothrombin time 22 sec* (control 18 sec) *Capillary resistance* 5 petechiae Fibrinogen 0 29 % Serum proteins 6 9 % Albumin 5 4 % Globulin 1 5 %

Present investigations Quick s prothrombin time 20 20 20 sec (control 19 sec) Spontaneous clotting time at 37 C 68 min Recalcification time in dilute plasma 10 min 30 sec *The thrombin generation test showed negligeable thrombin generation within 16 min (Curve A)*



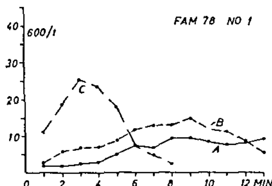
Curve B Addition of 0 2 ml of adsorbed bovine plasma *The thrombin generation normal* Recalcification time 3 min

Curve C Addition of 0 2 ml of normal serum *The thrombin generation poor Recalcification time 5 min*

Curves D and E illustrate the thrombin generation after addition of readsorbed serum and heated *readsorbed* serum respectively

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Present investigations Quick s prothrombin time 19 19 19 sec (control 16 sec) Thrombocytes 454 000 per μl of plasma Recalcification time in dilute plasma 5 min 22 sec The thrombin generation test showed a very low and very slowly rising thrombin concentration (Curve A)



Curve B Addition of 0 2 ml of normal serum Little improvement of the thrombin generation Recalcification time 3 min

Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recal ification time 1 min 20 sec

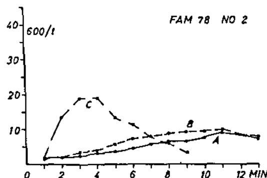
Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

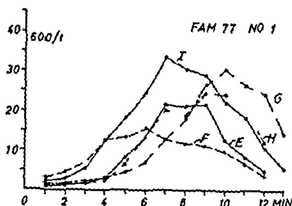
2 Born 1945 A bleeding tendency manifested itself from early infancy by frequent subcutaneous haematomas When he began to crawl haemarthroses occurred chiefly in the knees He has also had bleedings into the ankles and into a single finger joint The patient has received several transfusions The left knee is stiff in ?0 degrees flexion X ray of the knees ankles and left elbow (1953) revealed minor destructions of the borders of the joints and halisteresis 3 years old he was submitted to adenotomy Transfusion was necessary on account of excessive bleeding At the age of 4 he had a tooth knocked out This was followed by prolonged bleeding Another transfusion had to be given

Blood analyses (The Rigshospitalet Paediatric Dept) 1953 Thrombocytes 296 000–412 000 Prothrombin time 20 sec (control 18 sec) Bleeding time 7 min Clotting time 22–45 min

(The Vejle Amts og Bys Sygehus) Clotting time 18 min

Present investigations Quick s prothrombin time 18 18 18 sec (control 18 sec) Thrombocytes 459 000 per μl of plasma Recalcification time in dilute plasma 4 min 45 sec The thrombin generation test showed negligeable thrombin generation (Curve A)





Curve E Similar conditions were found after addition of 1 ml of the patient's own four times washed platelets (500 000 per μl of suspension) Recalcification time 4 min 38 sec

Curve F Freezing of the plasma with its normal content of platelets accelerated the thrombin generation and its inactivation but the thrombin concentrations attained were very low Recalcification time 2 min

Curve G Addition of 0 2 ml of plasma from patient no 4 fam 54 (AHF deficiency) The thrombin generation delayed Recalcification time 6 min

Curve H Addition of 0 2 ml of plasma from patient no 6 fam 8 (Christmas disease) The thrombin generation still delayed Recalcification time 5 min 15 sec

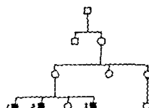
Curve I Simultaneous addition of the two latter plasma samples effected a perfectly normal thrombin generation Recalcification time 3 min 45 sec

Diagnosis Deficiency of the antihaemophilic factor plus Christmas factor

Comments

As the clotting defect could be corrected by adsorbed plasma and the serum factors seperately it is probably a question of a slight defect This is further borne out by the fact that considerable though delayed amounts of thrombin are formed in the patient's plasma without addition of test substrates

Family 78

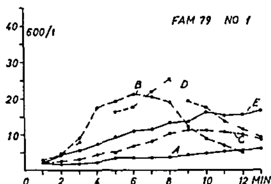


1 Born 1943 Proposatus From birth the patient displayed a liability to subcutaneous haematomas When he began to crawl bleedings occurred into the ankles and knees These have left no permanent joint deformities The patient has had one episode of haematuria in relation to an injury 3 years old he was submitted to appendicectomy In relation to this he had to have numerous blood transfusions

Blood analyses (The Vejle Amts og Bys Sygehus Med Dept) Clotting time 8–30 min Bleeding time 6–10 min Thrombocytes 415 000 Prothrombin time 24 sec (control 25 sec)

right knee He has had a few episodes of haematuria but never gastro intestinal bleedings At the age of 9 the patient s right eye was destroyed by a posttraumatic haemorrhage

Blood analyses Quick s prothrombin time 19 18 18 sec (control 19 sec) Thrombocytes 208 000 per μl of plasma Recalcification time in dilute plasma 13 min 20 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 15 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 5 min 15 sec

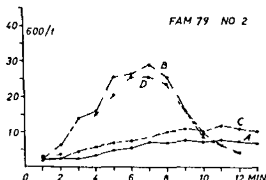
Curve D Addition of 0 2 ml of plasma from a patient with Christmas disease (fam 6 no 2) The thrombin generation normal Recalcification time 3 min 30 sec

Addition of plasma from a patient with AHF deficiency (fam 10 no 2) The thrombin generation poor Recalcification time 3 min 45 sec (Curve E)

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

2 Born 1943 A bleeding tendency was first noticed when the patient was 2 years old manifesting itself by profuse gingival haemorrhage in relation to dentition The patient has since been liable to bleedings into the skin spontaneous as well as following minor injuries He has had repeated haemorrhages into the ankles Since the age of 3 there has been continuously aggravating talipes equinus of the right foot A life threatening haemorrhage following blood sampling from the ear lobe necessitated transfusion He has never had haematemesis melaena epistaxis nor haematuria

Blood analyses (The Rigshospitalet Paediatric Dept) Prothrombin 28 sec (control [illegible] sec) Clotting time 1 hour 35 min Thrombocytes 111 000 Bleeding time 6 min Capillary resistance (80 mm mercury for 5 min) No petechiae Fibrinogen 0 36 %



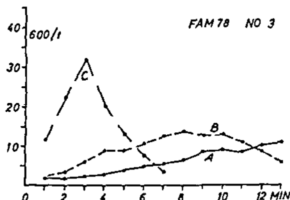
Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 3 min 45 sec
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 1 min

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

3 Born 1951 Since early infancy the patient has been liable to profuse bleedings into the skin Nearly 2 years old he was admitted to hospital on account of bleeding of 2 days duration from the tongue after a bite

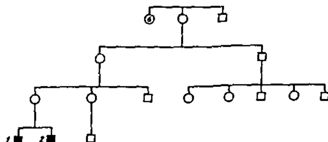
Blood analyses (The Vejle Amts og Bys Sygehus) Bleeding time 4 min 35 sec

Present investigations Quick s prothrombin time 19 19 20 sec (control 18 sec) Thrombocytes 419 000 per μl of plasma Recalcification time in dilute plasma 5 min 30 sec The thrombin generation test showed a very poor thrombin generation (Curve A)



Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 3 min 15 sec
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 1 min
Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 79



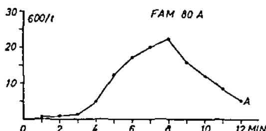
1 Born 1940 Propositus 18 months old the patient had a large extravasation of blood in one thigh There have been frequent subcutaneous and intramuscular haemorrhages since He has had several bleedings into the soft tissue of the neck but these have never caused a choking sensation He has had frequent bleedings into the elbows knees ankles toe joints and shoulders There is slightly limited mobility of the right elbow and the

Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 4 min
Curve C and D Addition of either 0 2 ml of normal serum or 0 2 ml of heated read sorbed serum gave a normal thrombin generation The corresponding recalcification times were 1 min 33 sec and 2 min 50 sec
Curve E. The thrombin generation normal in plasma with its normal content of platelets after this had been stored at —?0 C for 2 days Recalcification time 2 min 30 sec
Curve F A corresponding experiment with platelet poor plasma (6000 platelets per µl of plasma) Recal ification time 4 min 30 sec
Curve G 0 2 ml of the patient s plasma normalised the thrombin generation in plasma from a patient with both AHF and Christmas factor deficiency (fam 1 no 4) Recalcification time 2 min 45 sec

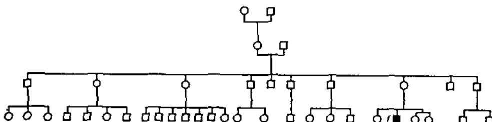
The patient s plasma could also normalise the thrombin generation in plasma lacking the AHF (fam 13) and plasma lacking the Christmas factor (fam 22)

Diagnosis Deficiency of the Hageman factor (the Hageman trait)

A Born 1930 There have never been any signs of a haemorrhagic diathesis
Blood analyses Quick s prothrombin time 20 ?0 20 sec (control 19 sec) Thrombocytes 668 000 per µl of plasma Recalcification time in dilute plasma 3 min 50 sec The thrombin generation test showed a normal thrombin generation (Curve A)



Family 82



The propositus is the only haemophiliac known in the family

1 Born 1938 Propositus The patient has bruised easily since his first year of life 18 months old he had a haemorrhage into his right knee joint He has since had several haemarthroses in the elbows knees and ankles The mobility of both knees is limited 1948 X ray of the right knee joint revealed grave arthrotic changes with deformation of the articular surfaces of the tibia and femur and in addition halisteresis of both femora The patient has had frequent episodes of epistaxis as well as a few of haematuria

Present investigations Quick's prothrombin time 19 20 20 sec (control 19 sec) Thrombocytes 644 000 per µl of plasma Clotting time for whole blood at 37 C 2 hours Recalcification time in dilute plasma 8 min 15 sec The thrombin generation test showed negligeable thrombin generation (Curve A)

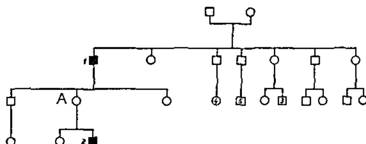
Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 15 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 4 min 55 sec

Curve D Addition of 0 2 ml of plasma from a patient with Christmas disease (fam 62 no 3) The thrombin generation normal Recalcification time 3 min 15 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

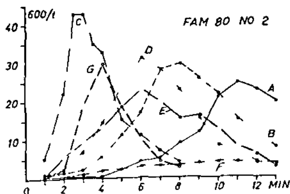
Family 80

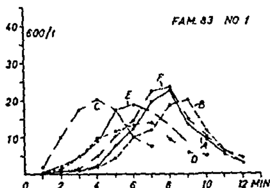


1 Born 1900 A bleeding tendency was manifest from early infancy The patient suffered from gingival haemorrhages on second dentition melaena haematuria intramuscular and subcutaneous bleedings prolonged haemorrhages from minor cuts and haemarthroses specially in the elbows The patient was in hospital several times on account of the haemophilic disease He died at home at the age of 48 apparently from coronary thrombosis

2 Born 1956 Propositus 6 months old the patient had a bleeding into the left ankle Since there have been several subcutaneous bleedings and haemarthroses localised in the ankles He had prolonged gingival haemorrhages on first dentition He has had neither haematuria melaena nor intramuscular bleedings

Blood analyses Clotting time (37 C) 1 hour 45 min Quick's prothrombin time 16 17 18 sec (control 16 sec) Thrombocytes 660 000 per µl of plasma Recalcification time in dilute plasma 5 min 53 sec The thrombin generation test showed a slow rise of the thrombin concentration after a lag period of 4–6 min (Curve A)





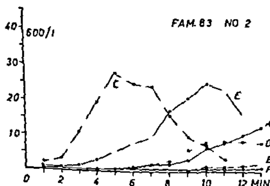
Curve B Addition of 0 2 ml of adsorbed bovine plasma No improvement of the thrombin generation. Recalcification time 4 min 10 sec
Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 1 min 20 sec
Curve D The thrombin generation likewise improved after addition of 0 2 ml of heated readsorbed serum though the improvement was less pronounced Recalcification time 2 min 50 sec
Curve E Addition of 0 2 ml of frozen platelet suspension (250 000 platelets per μl) The thrombin generation improved Recalcification time 2 min 40 sec
Curve F The thrombin generation did not improve after storage of the plasma with its normal content of platelets at −20 C Recalcification time 4 min 5 sec

Diagnosis Obs I Christmas disease

2 Born 1954 Propositus The patient has always bruised easily One year old he developed a large haematoma on the forehead after an injury 2 years old he had an intra abdominal haemorrhage attended by intense pain and intumescence under the right curvature He was given transfusion with a favourable effect on the acute case He has never had melaena haematemesis haematuria epistaxis gingival haemorrhage nor haemarthroses

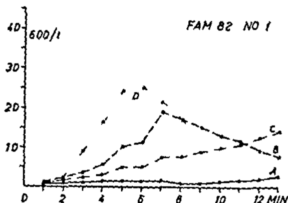
Blood analyses 1956 (The Rigshospitalet Paediatric Dept) Clotting time 18–46 min. Prothrombin time 19 sec (control 18 sec) Thrombocytes 274 000 Bleeding time 4 min Capillary resistance (Bexelius) 3 petechiae

Present investigations Quick s prothrombin time 19 19 19 sec (control 19 sec) Thrombocytes 230 000 per μl of plasma. Recalcification time in dilute plasma 7 min 45 sec The thrombin generation test showed a very poor thrombin generation (Curve A)



There has never been melaena nor haematemesis The patient was a factory worker but lost his job when it became known that he was a bleeder He now receives dis ablement benefit

Blood analyses Quick s prothrombin time 19 19 19 sec (control 19 sec) Thrombocytes 178 000 per µl of plasma Recalcification time in dilute plasma 11 min 30 sec The thrombin generation test showed neglıgeable thrombin generation (Curve A)



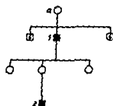
Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 30 sec

Curve C Addition of 0 2 ml of normal serum Little improvement of the thrombin generation Recalcification time 4 min 45 sec

Curve D No signs of an inhibitor Addition of 0 2 ml of fresh plasma resulted in fast generation and inactivation of thrombin Recalcification time 2 min 45 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 83



Information is available on the last few generations only because a) was placed out at the age of 4 years and knew nothing of her family

1 Born 1905 The patient has always bruised easily Between the ages of 2 and 24 he often had copious bleedings from the nose Tooth-cleaning was once followed by oozing gingival haemorrhage of several days duration The patient has never had haematuria haematemesis melaena nor haemarthroses Herniotomy in 1956 was followed by profuse bleeding into the scrotum and into the subcutaneous tissue of the abdomen A single transfusion had a favourable effect

Blood analyses 1956 (The Køge Sygehus) Prothrombin index 44–52–62 Bleeding time $6^1/_2$–5^1 –5 min Clotting time 7 7 $6^1/_2$ min Thrombocytes 104 000 195 000

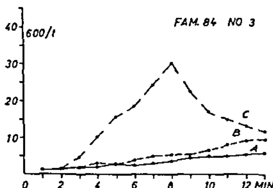
Present investigations (15 days after blood transfusion) Quick s prothrombin time 18 17 17 sec (control 18 sec) Thrombocytes 451 000 per µl of plasma Recalcification time in dilute plasma 4 min The thrombin generation test showed a steep rise and fall of the thrombin concentration but after a lag period of 4 min (Curve A)

Curve B Addition of 0 2 ml of serum The thrombin generation poor Recalcification time 4 min 45 sec
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 25 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

3 Born 1938 In early infancy the patient tended to bleed for a long time from scratches The first and second dentitions were associated with prolonged haemorrhages which often had to be treated by blood transfusions He has had frequent bleedings into the large joints Arthrodesis has been performed of the right knee joint He has never had haematemesis melaena nor haematuria He is liable to subcutaneous haemorrhages

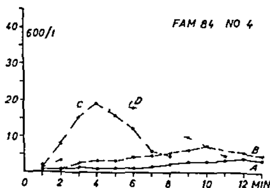
Blood analyses Quick s prothrombin time 18 18 18 sec (control 19 sec) Thrombocytes 343 00 per µl of plasma Recalcification time in dilute plasma 9 min 48 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of serum The thrombin generation poor Recalcification time 7 min 20 sec
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 15 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

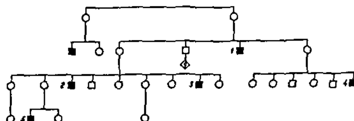
4 Born 1947 The patient has from his first year of life been liable to bruises and to prolonged bleedings from minor lesions There have been haemorrhages into the knees and elbows but these have not yet caused permanent changes He has had neither haematuria melaena nor haematemesis



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 11 min 52 sec
Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 2 min 30 sec
Curve D Addition of 0 2 ml of heated readsorbed serum The thrombin generation poor Recalcification time 7 min 10 sec
Curve E The thrombin generation improved somewhat after addition of a platelet suspension Recalcification time 4 min 30 sec
Curve F illustrates the course of the thrombin generation in frozen platelet poor plasma (3000 per μl) Recalcification time 18 min

Diagnosis Christmas factor deficiency (Christmas disease)
The above patient has since developed an inhibitor

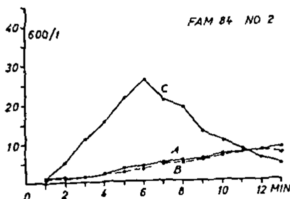
Family 84



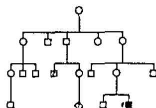
1 Is stated to have been a bleeder He died at the age of 48

2 Born 1929 Propositus From his first year of life the patient often had bleedings into the skin Later he also had frequent intramuscular bleedings The patient has had several episodes of severe epistaxis as well as prolonged bleedings after dental extractions necessitating numerous transfusions He has had melaena but never haematemesis There have been several episodes of haematuria He has had many haemarthroses especially in the elbows and knees where they have caused pronounced limitation of mobility The disease has grown somewhat milder within the past 3-4 years but the patient is still greatly handicapped by his disease and receives disablement benefit

Blood analyses Quick s prothrombin time 20 20 19 sec (control 19 sec) Thrombocytes 207 000 per μl of plasma Recalcification time in dilute plasma 7 min 45 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



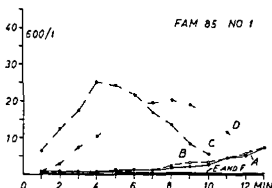
Family 85



The propositus is the only haemophiliac known in the family

1 Born 1946 Propositus 18 months old the patient had a gingival haemorrhage lasting 8 days after an injury The patient bled down to a HB ♂ of 29 and transfusion was required 3 years old he was again admitted to hospital on account of gingival haemorrhages He has bruised easily since early infancy 2 years old he bled for a long time after having bitten his tongue From the same age he has had haemarthroses chiefly in the ankles and elbows There are no permanent joint deformities The patient has never had haematuria haematemesis nor melaena Neither has he been troubled by epistaxis

Blood analyses Quick's prothrombin time 19 19 20 sec (control 19 sec) Thrombocytes 277 000 per μl of plasma Spontaneous clotting time at 37° C over 3¼ hours Recalcification time in dilute plasma 8 min 45 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 8 min 21 sec

Curve C Addition of 0 2 ml of normal serum The trombin generation normal Recalcification time 1 min 30 sec

Curve D After the plasma with its normal content of platelets had been frozen to −20 C for 24 hours the thrombin generation became normal Recalcification time 3 min 30 sec

Curve E No improvement of the thrombin generation in frozen platelet poor plasma Recalcification time over 17 min

Curve F Addition of 0 2 ml of heated readsorbed serum The thrombin generation poor Recalcification time 15 min

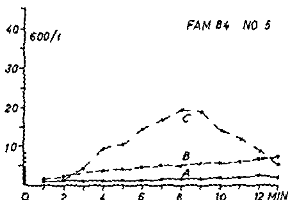
Diagnosis Christmas factor deficiency (Christmas disease)

Blood analyses Quick s prothrombin time 18 17 18 sec (control 19 sec) Thrombocytes 412 000 per µl of plasma Recalcification time in dilute plasma 9 min 45 sec The thrombin generation test showed negligeable thrombin generation (Curve A)

Curve B Addition of 0 2 ml of normal serum No essential improvement of the thrombin generation Recalcification time 4 min 40 sec
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 1 min 30 sec
Curve D No definite signs of an inhibitor Addition of 0 2 ml of normal plasma resulted in a fast rise and fall of the thrombin concentration Recalcification time 2 min

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)
On a previous investigation the above patient showed signs of a combined defect (AHF plus Christmas factor deficiency)

5 *Born 1948* A bleeding tendency was noticed from early infancy manifesting itself by large subcutaneous haematomas From the age of 6 he had bleedings into the elbows ankles and knees The latter joints have developed arthrotic changes The patient had several prolonged gingival haemorrhages on second dentition He has also had some episodes of epistaxis but never haematemesis haematuria nor melaena The patient has been given several transfusions
Blood analyses (The Viborg Amts og Skive Bysygehus Med Dept) Prothrombin index 94 % Fibrinogen 0 36 %
Present investigations Quick s prothrombin time 18 18 18 sec (control 19 sec) Thrombocytes 284 000 per µl of plasma Recalcification time in dilute plasma 14 min The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of normal serum No essential improvement of the thrombin generation Recalcification time 4 min 45 sec
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 30 sec

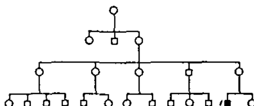
Diagnosis *Deficiency of the antihaemophilic factor (classical haemophilia)*

Curve F Freezing of platelet rich plasma gave a normal thrombin generation though after a lag period of 4 min Recalcification time 4 min 45 sec

Addition of plasma from patient no 3 fam 8 (Hageman trait) or from a patient with AHF deficiency (fam 105 no 1) normalised the thrombin generation

Diagnosis Christmas factor deficiency (Christmas disease)

Family 87

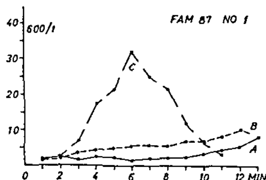


The propositus is the only family member known to have haemophilia

1 Born 1949 Propositus No unquestionable signs of haemophilia were seen until the age of 7 Extraction of a tooth then gave occasion to some bleeding which stopped spontaneously after about 2 hours At the same age a sudden unprovoked pain occurred in the right leg A few days later a large swelling developed on the lateral aspect of the right thigh At the same time the patient had become anaemic He has displayed no other signs of a haemorrhagic diathesis

Blood analyses 1956 (The Grenå Sygehus) Prothrombin index 95 % Bleeding time 2 min Clotting time 1 hour Thrombocytes 380 000 Fibrinogen 0 55 % Gothlin No petechiae The bone marrow showed active normocytic erythropoiesis and mild eosinophilia.

Present investigations Quick s prothrombin time 17 16 17 sec (control 17 sec) Thrombocytes 525 000 per µl of plasma Spontaneous clotting time at 37 C 1 hour 36 min Recalcification time in dilute plasma 19 min The thrombin generation test showed negligeable thrombin generation (Curve A)

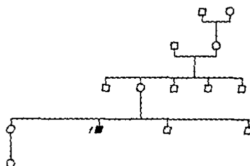


Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 4 min 45 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 58 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 86

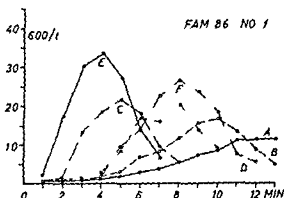


No cases of haemophilia are known beyond that of the *propositus*

1 Born 1925 Propositus A bleeding tendency has manifested itself since infancy by posttraumatic subcutaneous extravasations of blood The patient once bled for 8 days after dental extraction Blood transfusion was necessary In 1956 he bled for a long time after having bitten his tongue The patient has never had haemarthroses or renal haemorrhage Neither has he experienced gastro intestinal haemorrhage The patient owns a farm which he works himself without being particularly troubled by his disease

Blood analyses 1956 (The Rigshospitalet Dept A) Thrombocytes 315 000 Prothrombin time normal Bleeding time 1½–3 min Clotting time 3½ – 4–5 min Capillary resistance No petechiae

Present investigations Quick s prothrombin time 19 18 18 sec (control 19 sec) Owren s prothrombin proconvertin 90 % Thrombocytes 315 000 per µl of plasma The thrombin generation test showed a poor thrombin generation (Curve A) Recalcification time 6 min 45 sec

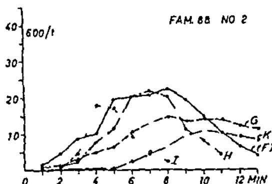


Curve B Addition of 0 2 ml of adsorbed bovine plasma Considerable improvement of the thrombin generation which however did not become quite normal Recalcification time 4 min 30 sec

Curve C Addition of normal serum The thrombin generation normal Recalcification time 2 min 30 sec

Curve D Addition of heated readsorbed serum The thrombin generation considerably improved Lag period about 4 min Recalcification time 4 min 20 sec

Curve E The patient s own serum normalised the thrombin generation in his own plasma Recalcification time 1 min 45 sec



Curve F Addition of 0 2 ml of fresh platelet suspension (380 000 platelets per μl of suspension) The thrombin generation normal Recalcification time 2 min 40 sec
Curve G Frozen platelet suspension did not improve the thrombin generation Recalcification time 4 min 40 sec
Curve H Storage of the patient s plasma with its normal content of platelets at −20 C for 72 hours effected a normal thrombin generation Recalcification time 3 min 20 sec
Curve I Using platelet poor plasma (7000 platelets per μl) the thrombin generation likewise became normal Recalcification time 3 min 30 sec

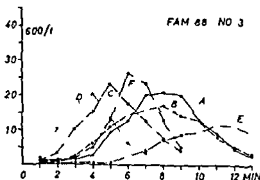
9 months before the above investigation the thrombin generation was found to be as illustrated by curve K The recalcification time was 5 min 50 sec

The patient s plasma normalised the thrombin generation in plasma from a Christmas patient (fam 85) but not that in plasma from a patient lacking the AHF (fam 13 no 5)

Diagnosis Slight deficiency of the antihaemophilic factor

3 Born 1935 The patient s bleeding tendency was first noticed when at the age of 2–3 years he was submitted to adeno and tonsillotomy There was prolonged oozing secondary haemorrhage which required admission to hospital The patient bruises easily and wounds give prolonged secondary bleeding He has never had haemarthroses haematuria melaena haematemesis nor intramuscular haemorrhages Until the age of 14 he had frequent episodes of epistaxis The patient who is an unskilled labourer is troubled but little by his disease He is never absent from his work on account of this He has never been given transfusions

Blood analyses 1954 (The Hornsyld Sygehus) Bleeding time 3 min 10 sec Thrombocytes 298 000 Clot retraction 4 min 15 sec



Family 88

2 Born 1875 39 years old the patient was submitted to herniotomy allegedly with no abnormal bleeding At the age of 41 he bled for a long time after traumatic amputation of a finger The patient thinks that this was the first time he became aware of his disease 45 years old he was operated on for haemorrhoids allegedly with no abnormal bleeding phenomena 64 years old he was operated on for Graves disease There was prolonged bleeding from the wound which had to be revised a few times The patient was then given transfusions The patient has had episodes of prolonged epistaxis and several oozing gingival haemorrhages lasting a week after dental extractions He bruises easily The patient has had a few episodes of melaena without an ulcer having been demonstrable but never haemarthroses nor haematuria

The patient has never felt particularly handicapped by his disease He is a blacksmith by profession and managed his work without difficulty He was in the Boer War as a private He now receives an old age pension

Blood analyses 1953 (The Horsens Kommunehospital) Capillary resistance No petechiae Bleeding time 4 min Clotting time 3 min Thrombocytes 245 000

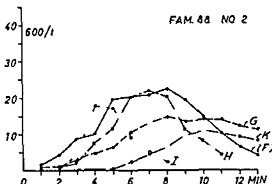
Present investigations Quick s prothrombin time 19 18 18 sec (control 19 sec) Prothrombin proconvertin according to Owren 80 % Thrombocytes 142 000 per μl of plasma Recalcification time in dilute plasma 4 min 15 sec The thrombin generation test showed a slow rise of the thrombin concentration (Curve A)

Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal the thrombin concentrations higher The lag period was about 4 min however Recalcification time 4 min 35 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 2 min 45 sec

Curve D Similar conditions were found after addition of 0 2 ml of the patient s own serum stored for nearly 12 months Recalcification time 2 min 45 sec

Curve E Addition of 0 2 ml of heated readsorbed normal serum The thrombin generation normal Recalcification time 3 min 35 sec



Curve F Addition of 0 2 ml of fresh platelet suspension (380 000 platelets per µl of suspension) The thrombin generation normal Recalcification time 2 min 40 sec

Curve G Frozen platelet suspension did not improve the thrombin generation Recalcification time 4 min 40 sec

Curve H Storage of the patient s plasma with its normal content of platelets at −20 C for 72 hours effected a normal thrombin generation Recalcification time 3 min 20 sec

Curve I Using platelet poor plasma (7000 platelets per µl) the thrombin generation likewise became normal Recalcification time 3 min 30 sec

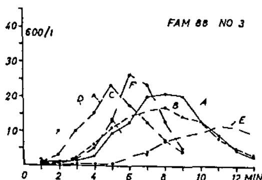
9 months before the above investigation the thrombin generation was found to be as illustrated by curve K The recalcification time was 5 min 50 sec

The patient s plasma normalised the thrombin generation in plasma from a Christmas patient (fam 85) but not that in plasma from a patient lacking the AHF (fam 13 no 5)

Diagnosis Slight deficiency of the antihaemophilic factor

3 Born 1935 The patient s bleeding tendency was first noticed when at the age of 2–3 years he was submitted to adeno and tonsillotomy There was prolonged oozing secondary haemorrhage which required admission to hospital The patient bruises easily and wounds give prolonged secondary bleeding He has never had haemarthroses haematuria melaena haematemesis nor intramuscular haemorrhages Until the age of 14 he had frequent episodes of epistaxis The patient who is an unskilled labourer is troubled but little by his disease He is never absent from his work on account of this He has never been given transfusions

Blood analyses 1954 (The Hornsyld Sygehus) Bleeding time 3 min 10 sec Thrombocytes 298 000 Clot retraction 4 min 15 sec



Present investigations Quick s prothrombin time 23 24 21 sec (control 19 sec) Prothrombin proconvertin (Owren) 120 % Thrombocytes 282 000 per µl of plasma Recalcification time in dilute plasma 4 min 20 sec The thrombin generation test showed a steep rise and fall of the thrombin concentration after a lag period of scarcely 4 min (Curve A)

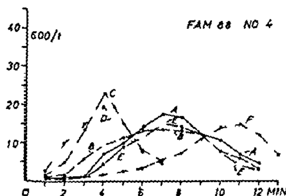
Curve B Addition of 0 2 ml of adsorbed bovine plasma reduced the lag period Recalcification time 3 min 30 sec
Curve C Addition of 0 2 ml of normal serum or of 0 2 ml of readsorbed serum (Curve D) effected a steep rise and fall of the thrombin concentration Recalcification times 2 min 20 sec and 2 min 10 sec
Curve E Addition of 0 2 ml of heated readsorbed normal serum The thrombin generation poor Recalcification time 6 min 10 sec
Curve F Freezing of the plasma with its normal content of platelets gave some acceleration of the thrombin generation Recalcification time 4 min
The patient s plasma normalised the thrombin generation in plasma from a Christmas patient (fam 85 no 1) but not that in plasma from a patient with AHF deficiency (fam 13 no 5)

Diagnosis Slight deficiency of the antihaemophilic factor

4 Born 1937 At the age of 11 months the patient bled for a long time from a small crack in the lip 8 years old he bled for 2 weeks from a minor cut in the foot He has had a few posttraumatic bleedings into the knee joints but not into other joints He is not particularly liable to bruises An injury at the age of 18 was followed by epistaxis lasting 6 days He has never had haematuria haematemesis nor melaena The patient is an unskilled labourer and able to live by his job

Blood analyses 1956 (The Hornsyld Sygehus) Thrombocytes 200 000 Clotting time 5 min 30 sec Bleeding time 7 min Prothrombin time 40 sec (control 41 sec)

Present investigations Quick s prothrombin time 24 25 24 sec (control 19 sec) Prothrombin proconvertin (Owren) 120 % Thrombocytes 352 000 per µl of plasma Recalcification time in dilute plasma 4 min The thrombin generation test showed a normal thrombin generation (Curve A)



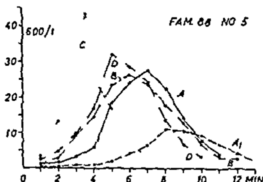
Curve B Addition of 0 2 ml of adsorbed bovine plasma caused some acceleration of the thrombin generation Recalcification time 3 min 20 sec
Curve C Addition of normal serum or of readsorbed serum (Curve D) resulted in a fast rise and fall of the thrombin concentration The recalcification times were 2 min 10 sec and 2 min 30 sec respectively
Curve E Addition of heated readsorbed serum did not improve the thrombin generation Recalcification time 4 min 30 sec

The patient s plasma improved the thrombin generation in AHF-deficient plasma (fam 13 and fam 59) but did not render it normal (Curve F) whereas it normalised the thrombin generation in plasma from a Christmas patient (fam 85)

Diagnosis Slight deficiency of the antihaemophilic factor

5 Born 1940 Tonsillectomy and adenotomy at the age of 2 years were followed by oozing haemorrhage lasting about 2 weeks The patient is liable to prolonged epistaxis After a dental extraction he bled for 11 days He bruises easily and the bruises are visible for a long time He has had a single haemorrhage into one knee The resulting changes persisted for about 2 months There are no permanent changes He has never had haematuria haematemesis nor melaena No blood transfusion has ever been given The patient is a compositor s apprentice and is not particularly handicapped by his disease

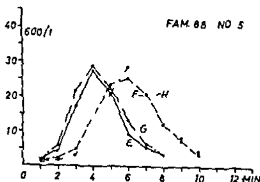
Blood analyses Quick s prothrombin time 19 18 18 sec (control 19 sec) Prothrombin proconvertin (Owren) 60 % Thrombocytes 453 000 per µl of plasma Recalcification time in dilute plasma 3 min 50 sec The thrombin generation test showed a normal thrombin generation after a lag period of just over 3 min (Curve A) 9 months previously the thrombin generation was as illustrated in curve A_1 Recalcification time 5 min 45 sec



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min

Curve C Addition of 0 2 ml of normal serum effected a very steep rise and fall of the thrombin concentration Recalcification time 1 min 55 sec

Curve D Addition of 0 2 ml of fresh platelet suspension (380 000 platelets per µl of suspension) likewise gave a very steep rise and fall of the thrombin concentration Recalcification time 2 min 30 sec



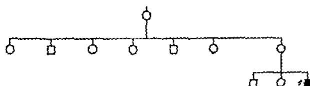
Curve E After storage of the patient's plasma at −20 C for 72 hours the thrombin concentration rose and fell at a very fast rate Recalcification time 2 min 30 sec

Curve F Addition of 0 2 ml of heated readsorbed serum also accelerated the thrombin generation Recalcification time 3 min 45 sec

The patient's plasma normalised the thrombin generation in plasma from a Christmas patient (fam 43) as well as in plasma from a patient with AHF deficiency (fam 84) See curves G and H respectively

Diagnosis Deficiency of the Hageman factor

Family 90

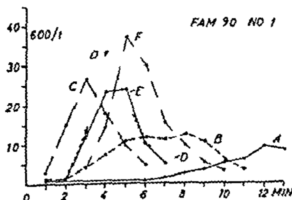


The propositus is the only haemophiliac known in the family

I Born 1942 Propositus 10 months old the patient cut his finger on a razor blade There was very prolonged oozing haemorrhage from the cut About the age of 18 months a large haematoma developed in the scrotum after a fall 2½ years old he had prolonged oozing haemorrhage from a bite in the border of the tongue Two large haematomas developed on the upper surface of the tongue These were removed surgically and the patient was given two transfusions From the age of 3–4 he had frequent haemorthroses comprising particularly the ankles and knees as well as the right wrist The patient bruises very easily On second dentition there were prolonged oozing haemorrhages The patient has occasionally had attacks of acute abdominal pain but never haematemesis melaena nor haematuria The disease has grown somewhat milder within the past 2–3 years though the patient is still liable to subcutaneous haemorrhages and bleedings into the joints The latter have left no permanent joint deformities

Blood analyses 1951 (The Rigshospitalet Paediatric Dept) Thrombocytes 134 000 *Prothrombin time 29 sec (control 29 sec) Serum protein 7 %* *Serum albumin* 4 8 % Fibrinogen 0 22 % Bleeding time 3½ min Capillary resistance 80 mm for 5 min No *petechiae*

Present investigations Clotting time (Andrassen Burker) 6 min –18 min Quick's prothrombin time 15 15 15 sec (control 17 sec) Thrombocytes 175 000 per µl of plasma Recalcification time in dilute plasma 8 min The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma effected considerable improvement of the thrombin generation the concentration of which rose and fell rather *quickly Recalcification time 2 min 45 sec*

Curve C Addition of 0 2 ml of normal serum caused a very steep rise and fall of the thrombin concentration Recalcification time 1 min

Curve D Addition of 0 2 ml of heated readsorbed serum The thrombin generation normal *Recalcification time 2 min 40 sec*

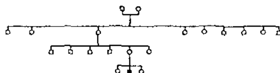
Curve E The thrombin generation in the patient s platelet containing plasma was normal after storage at −20 for 72 hours Recalcification time 2 min 25 sec

Curve F shows that the thrombin generation was normal in the patient s platelet poor plasma (?5 000 platelets per μl) after this had been stored at −20 C for 11 months Recalcification time 3 min 50 sec

The patient s plasma normalised the thrombin generation in AHF-deficient plasma (fam 84) and in plasma lacking the Christmas factor (fam 22)

Diagnosis Deficiency of the Hageman factor

Family 91



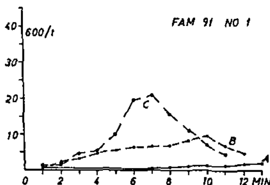
No other cases of haemophilia are known in the family

1 Born 1949 Propositus 18 months old he had a prolonged haemorrhage from a superficial cut in a finger pulp Dentition was not associated with bleeding From the age of 2 years he fairly often had swollen knees and ankles as well as accumulation of blood in the hip joints There are no permanent joint deformities The patient bruises easily Periodically there have been frequent episodes of epistaxis He has never had haematemesis nor melaena but one episode of mild haematuria

Blood analyses 1953 (The Rigshospitalet Paediatric Dept) Clotting time 20 min Bleeding time 3 min 30 sec Thrombocytes 272 000 Bexelius 4 petechiae Prothrombin time 24 sec (control 18 sec) Fibrinogen 0 33 %

1956 (The Centralsygehuset Slagelse Med Dept) Prothrombin proconvertin (Owren) 118 % Clotting time 6 min 30 sec *Bleeding time about 24 hours*

Present investigations Quick s prothrombin time 20 21 21 sec (control 19 sec) Thrombocytes 193 000 per μl of plasma Recalcification time in dilute plasma 7 min 40 sec The thrombin generation test showed no thrombin generation (Curve A)

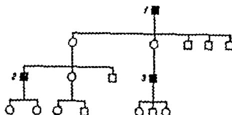


Curve B Addition of 0 2 ml of normal serum Little improvement of the thrombin generation Recalcification time 3 min 15 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 15 sec

Diagnosis *Deficiency of the antihaemophilic factor (classical haemophilia)*

Family 92

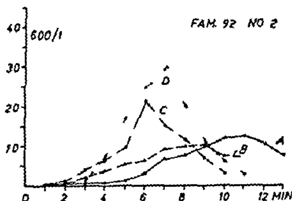


Several members of the family live in Germany

1 Haemophilic He died 1936 in Germany

2 Born 1919 Propositus The patient has always been liable to large ecchymoses after injuries Dental extractions were three times followed by very prolonged bleedings which necessitated transfusions The patient has never had haemarthroses melaena haematemesis haematuria nor epistaxis He is a grocer and manages well He does not receive disablement benefit

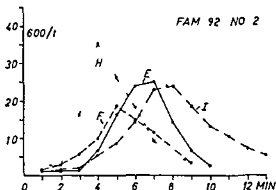
Blood analyses Quick s prothrombin time 20 20 21 sec (control 19 sec) Thrombocytes 215 000 per µl of plasma Recalcification time in dilute plasma 5 min 40 sec The thrombin generation test showed a poor thrombin generation after a lag period of 5 min (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma *Some acceleration of the thrombin generation* Recalcification time 3 min 40 sec

Curve C *Addition* of 0 2 ml of normal serum The thrombin generation normal Recalcification time 3 min 20 sec

Curve D Similar conditions were found after addition of heated readsorbed normal serum Recalcification time 3 min 30 sec



Curve E The thrombin generation was normal after the platelet-containing plasma had been stored at −20 C for 11 days Recalcification time 4 min
Curve F Addition of normal frozen platelet suspension gave a normal thrombin generation Recalcification time 3 min
Curve H Storage of platelet poor patient plasma (5000 platelets per µl of plasma) at −20 C for 9 months effected a normal thrombin generation Recalcification time 2 min 35 sec
Curve I shows the thrombin generation in the patients plasma 10 months after the first investigation Recalcification time 4 min 30 sec The thrombin generation seemed to be normal

The patient s plasma normalised the thrombin generation in plasma from a Christmas patient (fam 2?) but not that in plasma lacking the AHF (fam 84) or with slight AHF deficiency (fam 41 no 2)

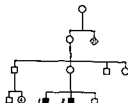
Diagnosis Slight deficiency of the antihaemophilic factor

Comments

It is remarkable that this patient s plasma which according to the cross tests with plasma samples having known defects lacks the AHF was found in the substitution experiments to be corrected the best by test substrates prepared from serum On a subsequent investigation however an equally good correction was obtained with adsorbed bovine plasma Further it may be pointed out that the patient s own serum was able to correct his clotting defect a fact also noticed in family 88

3 Haemophilic He lives in Germany

Family 93

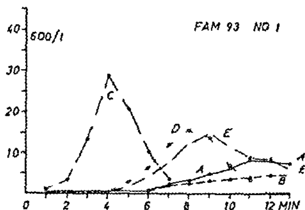


Six generations back there is said to have been a boy with a bleeding tendency

1 Born 1946 18 months old the patient bled for a long time from the upper lip after contusion He has often had mucosal haemorrhages lasting a week in the oral cavity Dental extractions were followed by prolonged oozing haemorrhages of up to one week s

duration He has never had haematuria haemarthroses haematemesis nor melaena The patient is liable to subcutaneous haematomas He is troubled but little by his disease

Blood analyses Quick s prothrombin time 19 19 18 sec (control 17 sec) Thrombocytes 347 000 per ul of plasma Recalcification time in dilute plasma 7 min 50 sec The thrombin generation test (Curve A) showed a poor thrombin generation after a lag period of 6 min



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 7 min 30 sec

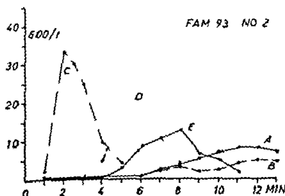
Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 2 min 30 sec

Curve D Storage at −20 C of the plasma with its normal content of platelets improved the thrombin generation the concentration now rising considerably after a lag period of 4 min Recalcification time 5 min 50 sec

Curve E Heated readsorbed serum improved the thrombin generation after a lag period of 4 min Recalcification time 6 min

Diagnosis Christmas factor deficiency (Christmas disease)

2 Born 1948 Propositus The patient displayed no abnormal bleeding phenomena the first 5 years of life 5 years old he developed a haematoma on the lip after an injury Puncture of this resulted in prolonged oozing haemorrhage which necessitated admission to hospital Two transfusions were required before haemostasis was obtained There was prolonged bleeding from a bite in the tongue The patient was in hospital for 4 weeks on account of this He has had many episodes of prolonged epistaxis but never haemarthroses haematuria haematemesis nor melaena He bruises very easily He has had no gingival haemorrhages



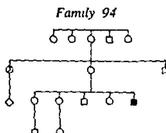
Blood anal ses 1955 (The Alborg Amtssygehus Med Dept) Thrombocytes 450 000–373 000 Bleeding time 1½–2½ min Clotting time 9 min Prothrombin time 103–61 % Fibrin 287 mg % Recalcification time normal No signs of an inhibitor of the plasma thromboplastin Capillary resistance No petechiae

Present investigations Quick s prothrombin time 18 18 19 sec (control 17 sec) Recalcification time in dilute plasma 7 min 15 sec The thrombin generation test showed negligeable thrombin generation (Curve A)

Curve B Addition of 0 ? ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 6 min
Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 1 min 30 sec
Curve D Addition of heated readsorbed serum likewise improved the thrombin generation but after a longer lag period (just over 3 min) Recalcification time 4 min
Curve E Storage of the patient s plasma with its normal content of platelets at –20 C for 48 hours gave little improvement of the thrombin generation. Recalcification time 5 min 0 sec

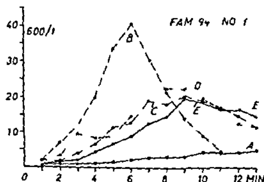
Diagnosis Christmas factor deficiency (Christmas disease)

Family 94



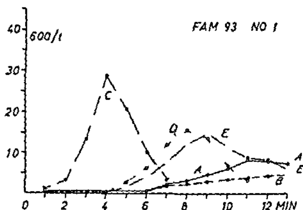
The propositus is the only haemophiliac known in the family

1 Born 1941 Propositus 18 months old the patient fell and hurt his chin which bled for so long that admission to hospital was necessary He has often had epistaxis and subcutaneous haematomas There have been several bleedings into the knees but these have not left permanent changes He has never had haematuria haematemesis nor melaena On second dention frequent prolonged gengival haemorrhages were experienced The patient has received several blood transfusions The disease seems to have grown milder in the course of years The patient is apprenticed to a painter and manages well He has experienced no abnormal bleeding phenomena the past 18 months



duration He has never had haematuria haemarthroses haematemesis nor melaena The patient is liable to subcutaneous haematomas He is troubled but little by his disease

Blood analyses Quick s prothrombin time 19 19 18 sec (control 17 sec) Thrombocytes 347 000 per ul of plasma Recalcification time in dilute plasma 7 min 50 sec The thrombin generation test (Curve A) showed a poor thrombin generation after a lag period of 6 min



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 7 min 30 se

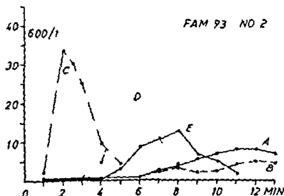
Curve C Addition of 0 . ml of normal serum The thrombin generation normal Recalcification time 2 min 30 sec

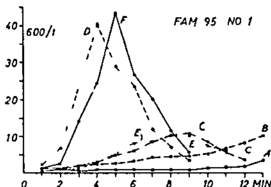
Curve D Storage at − 20 C of the plasma with its normal content of platelets improved the thrombin generation the concentration now rising considerably after a lag period of 4 min Recalcification time 5 min 50 sec

Curve E Heated readsorbed serum improved the thrombin generation after a lag period of 4 min Recalcification time 6 min

Diagnosis Christmas fa tor deficiency (Christmas disease)

2 Born 1948 Propositus The patient displayed no abnormal bleeding phenomena the first 5 years of life 5 years old he developed a haematoma on the lip after an injury Puncture of this resulted in prolonged oozing haemorrhage which necessitated admission to hospital Two transfusions were required before haemostasis was obtained There was prolonged bleeding from a bite in the tongue The patient was in hospital for 4 weeks on account of this He has had many episodes of prolonged epistaxis but never haemarthroses haematuria haematemesis nor melaena He bruises very easily He has had no gingival haemorrhages





Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 2 min 45 sec
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 3 min 15 sec
Curve D Addition of 0 2 ml of adsorbed bovine plasma as well as 0 2 ml of normal serum The thrombin generation normal Recalcification time 2 min 25 sec
Curve E Addition of 0 2 ml of adsorbed bovine plasma to the patient s plasma stored with its normal content of platelets at −20 C for 2 months did not improve the thrombin generation more than addition of bovine plasma to fresh patient plasma Recalcification time 4 min
Curve F There were no signs of an inhibitor Addition of 0 2 ml of normal plasma effected fast generation and inactivation of thrombin Recalcification time 2 min 30 sec

Diagnosis Deficiency of the antihaemophilic factor plus Christmas factor (combined haemophilia)

Family 96

No information is available on the family the patient being a stepchild

1 Born 1944 Propositus The patient has since birth been liable to large bruises On second dentition there were prolonged gingival haemorrhages He has had frequent bleedings into the knees and elbows The left knee is almost stiff He has never had haematuria but a single episode of melaena He has not been troubled by epistaxis The patient has been given many blood transfusions e g in relation to each dentition Bleedings from wounds ar very difficult to stop Haemostasis is generally obtained only after transfusions of which he has received about 100

Blood analyses 1946 (The Rigshospitalet Paediatric Dept) Prothrombin time 18 sec (control 18 sec) Bleeding time 10 min Clotting time $1^{1}-^{?}$ hours Thrombocytes 399 000 Capillary resistance 3 petechiae Fibrinogen 0 34

Present investigations Quick s prothrombin time 18 17 17 sec (control 18 sec) Thrombocytes 478 000 per µl of plasma Recalcification time in dilute plasma 9 min 50 sec The thrombin generation test showed negligeable thrombin generation (Curve A)

Blood analyses 1957 (The Rigshospitalet Dept B) Thrombocytes 273 000 Prothrombin 62 % Clotting time 26 min Bleeding time 6 min

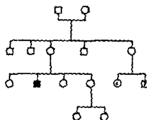
Present investigations Quick s prothrombin time 17 17 16 sec (control 17 sec) Thrombocytes 580 000 per μl of plasma Recalcification time in dilute plasma 7 min 45 sec The thrombin generation test showed negligeable thrombin generation (Curve A)

Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min
Curve C Addition of 0 2 ml of normal serum effected a slower thrombin generation (maximum thrombin concentration at 9 min) Recalcification time 3 min 45 sec
Curve D Addition of 0 2 ml of readsorbed normal serum gave a still better thrombin generation than serum Recalcification time 3 min 50 sec
Curve E 0 2 ml of heated readsorbed normal serum effected little improvement of the thrombin generation Recalcification time 4 min 45 sec

The patient s plasma could not normalise the thrombin generation in AHF deficient plasma (fam 84 no 5) but normalised that in plasma lacking the Christmas factor (fam 85)

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

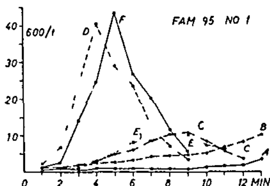
Family 95



1 Born 1913 Propositus A bleeding tendency was noticed from early infancy Especially the patient has been troubled by haemarthroses chiefly in the knees ankles and elbows The knee joints could be bent no more than about 25 degrees Both elbows presented 30 degrees extension defect The patient bruised easily and had frequent episodes of prolonged epistaxis but never gingival haemorrhages He never had haematuria but melaena and haematemesis The frequency of bleeding phenomena decreased somewhat with increasing years The patient received disablement benefit In 1954 a cyst was detected in the left ilium Microscopy of tissue from this (1956) revealed osteitis fibrosa like changes and myositis ossificans of the muscles surrounding the hip joint presumably a sequel of previous haemorrhages In 1956 the patient was admitted to hospital owing to profuse bleeding from a large haematoma in the region of the right hip On incision there were found large malodorous haematoma masses and pus as well as bleeding from the bony cyst The patient died from anaemia in spite of frequent transfusions Post mortem diagnosis Excessive anaemia of the organs Osteofibrosis of the pelvis and the proximal end of the left femur Arthrosis of the left knee Emaciation Myositis ossificans of the adductors of the left thigh (haemophilia)

Blood analyses 1954 (The Viborg Amts og Bys Sygehus) Clotting time 10–12 min Bleeding time 6 min Prothrombin index 100

Present investigations Quick s prothrombin time 17 16 17 sec (control 17 sec) Thrombocytes 228 000 per μl of plasma Recalcification time in dilute plasma 12 min 30 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 2 min 45 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation poor Recalcification time 3 min 15 sec

Curve D Addition of 0 2 ml of adsorbed bovine plasma as well as 0 2 ml of normal serum The thrombin generation normal Recalcification time 2 min 25 sec

Curve E Addition of 0 2 ml of adsorbed bovine plasma to the patient s plasma stored with its normal content of platelets at −20 C for 2 months did not improve the thrombin generation more than addition of bovine plasma to fresh patient plasma Recalcification time 4 min

Curve F There were no signs of an inhibitor Addition of 0 2 ml of normal plasma effected fast generation and inactivation of thrombin Recal ification time 2 min 30 sec

Diagnosis Deficiency of the antihaemophilic factor plus Christmas factor (combined haemophilia)

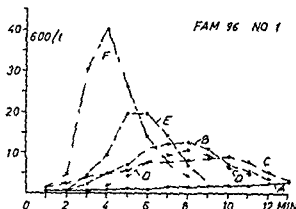
Family 96

No information is available on the family the patient being a stepchild

1 Born 1944 Propositus The patient has since birth been liable to large bruises On second dentition there were prolonged gingival haemorrhages He has had frequent bleedings into the knees and elbows The left knee is almost stiff He has never had haematuria but a single episode of melaena He has not been troubled by epistaxis The patient has been given many blood transfusions e g in relation to each dentition Bleedings from wounds ar very difficult to stop Haemostasis is generally obtained only after transfusions of which he has received about 100

Blood analyses 1946 (The Rigshospitalet Paediatric Dept) Prothrombin time 18 sec (control 18 sec) Bleeding time 10 min Clotting time 1 ½–4 hours Thrombocytes 399 000 Capillary resistance 3 petechiae Fibrinogen 0 34 %

Present investigations Quick s prothrombin time 18 17 17 sec (control 18 sec) Thrombocytes 478 000 per µl of plasma Recalcification time in dilute plasma 9 min 50 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma gave considerable improvement of the thrombin generation Recalcification time 3 min 30 sec

Curve C Addition of 0 2 ml of normal serum Some improvement of the thrombin generation Recalcification time 2 min 30 sec

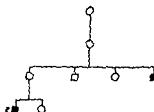
Curve D Addition of 0 2 ml of adsorbed bovine plasma to platelet rich plasma stored at −20 C accelerated the thrombin generation but the concentrations obtained were rather low Recalcification time 4 min 15 sec

Curve E Addition of 0 2 ml of adsorbed bovine plasma as well as 0 2 ml of heated readsorbed serum The thrombin generation normal Recalcification time 3 min 20 sec

Curve F The thrombin generation became normal after addition of 0 2 ml of adsorbed bovine plasma to the patient s platelet poor plasma (9000 per µl) after this had been stored at −20 C for 14 months Recalcification time 2 min. 7 sec.

Diagnosis Deficiency of the antihaemophilic factor plus freezing/serum factor

Family 97



Only four generations of the family are known the maternal grandmother of the propositus being an adopted child

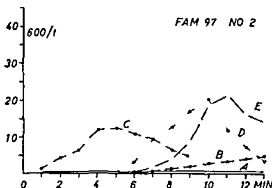
1 Born 1944 The patient died in 1951 from haemorrhage into the throat From the age of 6 months he had frequent subcutaneous haematomas often of apparently spontaneous origin He once bled for a long time from the ear lobe after blood sampling Scarcely 7 years old the patient bled from the left tonsil after having had an orthrodontic clasp fixed The patient was admitted to hospital greatly shocked and anaemic He died shortly after admission during blood transfusion Extensive extravasation of blood was demonstrated submucously in the tongue in the pharynx as well as the left cheek and into the soft tissue of the neck Large ecchymoses were seen on the head and neck

Blood analyses 1946 (The Rigshospitalet Paediatric Dept) Thrombocytes 511 000 Prothrombin time 22 sec (control 18 se) Bleeding time over 12 hours–9 min Capillary resistance 80 mm for 3 min No petechiae Clot retraction 15–90 min Fibrinogen 0 39 %

2 Born 1950 Propositus From the age of 10 months a bleeding tendency manifested itself by bruises after injuries prolonged bleedings from minor cuts and frequent episodes of prolonged epistaxis One year old he developed a large haematoma on the forehead followed by orbital haematomas At the age of about one year he had melaena From the age of 5 there were frequent haemarthroses specially in the ankles knees and hips 5 years old he had profuse intramuscular haemorrhage into the right lower leg and ankle during which the Achilles tendom became necrosed There is limited mobility of the right ankle the right knee and the right hip The patient has never had haematuria

Blood analyses 1951 (The Rigshospitalet Paediatric Dept) Thrombocytes 162 000 Clotting time at 37 C 9 min 25 sec–10 min 15 sec Prothrombin time 20 sec (control 19 sec) Capillary resistance (70 mm for 4 min) No petechiae Bleeding time 3–7 min Fibrinogen 0 41 % Serum protein 7 3 %

Present investigations Clotting time (Burker Andreassen) 7 min to over 30 min Quick s prothrombin time 17 18 17 sec (control 17 sec) Thrombocytes 313 000 per µl of plasma Recalcification time in dilute plasma 18 min 35 sec The thrombin generation test showed no thrombin generation (Curve A)



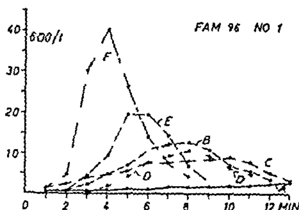
Curve B Addition of 0 2 ml of adsorbed bovine plasma No essential improvement of the thrombin generation Recalcifi ation time 8 min

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recal cification time 2 min 15 sec

Curve D The thrombin generation was somewhat improved but not normal in the pa tient s plasma stored with its normal content of platelets at –20 C for 10 months Recalcification time 7 min

Curve E Addition of 0 2 ml of heated readsorbed serum effected essential improvement of the thrombin generation after a lag period of about 8 min Recalcification time 7 min 20 sec

Diagnosis Christmas factor deficiency (Christmas disease)



Curve B Addition of 0 2 ml of adsorbed bovine plasma gave considerable improvement of the thrombin generation Recalcification time 3 min 30 sec

Curve C Addition of 0 2 ml of normal serum Some improvement of the thrombin generation Recalcification time 2 min 30 sec

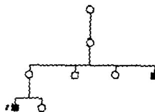
Curve D Addition of 0 2 ml of adsorbed bovine plasma to platelet rich plasma stored at −20 C accelerated the thrombin generation but the concentrations obtained were rather low Recalcification time 4 min 15 sec

Curve E Addition of 0 2 ml of adsorbed bovine plasma as well as 0 2 ml of heated readsorbed serum The thrombin generation normal Recalcification time 3 min 20 sec

Curve F The thrombin generation became normal after addition of 0 2 ml of adsorbed bovine plasma to the patient s platelet poor plasma (9000 per μl) after this had been stored at −20 C for 14 months Recalcification time 2 min 7 sec

Diagnosis Deficiency of the antihaemophilic factor plus freezing serum factor

Family 97



Only four generations of the family are known the maternal grandmother of the propositus being an adopted child

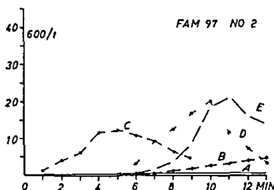
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Blood analyses 1951 (The Rigshospitalet Paediatric Dept) Thrombocytes 162 000 Clotting time at 37 C 9 min 25 sec – 10 min 15 sec Prothrombin time ?0 sec (control 19 sec) Capillary resistance (70 mm for 4 min) No petechiae Bleeding time 3–7 min Fibrinogen 0 41 % Serum protein 7 3 %

Present investigations Clotting time (Burker Andreassen) 7 min to over 30 min Quick s prothrombin time 17 18 17 sec (control 17 sec) Thrombocytes 313 000 per µl of plasma Recalcification time in dilute plasma 18 min 35 sec The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 ∠ ml of adsorbed bovine plasma No essential improvement of the thrombin generation Recalcification time 8 min

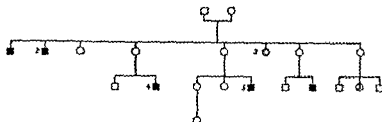
Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recal cification time ∠ min 15 sec

Curve D The thrombin generation was somewhat improved but not normal in the pa tient s plasma stored with its normal content of platelets at –20 C for 10 months Recalcification time 7 min

Curve E Addition of 0 2 ml of heated readsorbed serum effected essential improvement of the thrombin generation after a lag period of about 8 min Recalcification time 7 min 20 sec

Diagnosis Christmas factor deficiency (Christmas disease)

Family 98



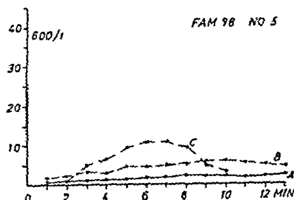
1 The patient bled to death at home about 12 months old

2 The patient died at home about 10 years old from gingival haemorrhage

3 The patient died in 1940 about 30 years old from loss of blood A bleeding tendency manifested itself from the age of 4 years by a liability to bruises gingival haemorrhages bleedings lasting several days from minor cuts and occasionally swelling of the joints with associated tenderness and pain On admission to hospital in 1926 (Tarm) her knee joints were swollen and she bled from the gums
Blood analyses Bleeding time 2¾ min

4 Born 1934 The patient bruised easily but displayed no other signs of a haemorrhagic diathesis He bled to death at the age of 2½ years after operation for phimosis

5 Born 1938 Propositus The patient has bruised easily since early infancy both in relation to injuries and spontaneously He has had several episodes of prolonged epistaxis as well as haemorrhages into the shoulders elbows and finger joints There are no permanent joint deformities The patient has had neither haematuria melaena nor haematemesis
Blood analyses Quick s prothrombin time 18 18 18 sec (control 18 sec) Thrombocytes 384 000 per ul of plasma Recalcification time in dilute plasma 7 min 45 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



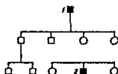
Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 2 min
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal The concentrations were not particularly high however Recalcification time 3 min

5 months later the experiment was repeated by adding 0 2 ml of bovine plasma to platelet poor (10 000 platelets) as well as to normal platelet rich plasma after storage at −20 C In both cases the thrombin concentration was found to rise and fall at a fast rate

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

6 Born 1952 He is stated to bruise easily
The author has had occasion to examine the blood of the first ancestress of family 98 No signs of a clotting defect were detectable

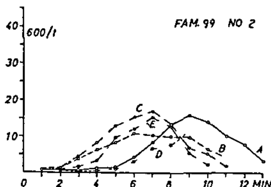
Family 99



1 Born 1873 The patient is believed to have had a very mild degree of haemophilia He has never been in hospital on account of this He died at the age of 79

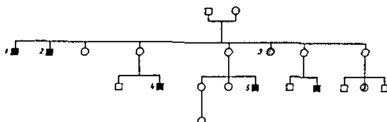
2 Born 1947 The patient has always bruised easily He has had several episodes of prolonged epistaxis Just over 12 months old he developed a large haematoma in the gum after an injury He has never had haemarthroses haematuria haematemesis nor melaena Neither has he had bleedings on second dentition The patient has never been given a blood transfusion He has always been fully able to attend school

Blood analyses Quick s prothrombin time 19 20 20 sec (control 19 sec) Thrombo cytes 379 000 per μl of plasma Recalcification time in dilute plasma 5 min 30 sec The thrombin generation test showed a rather fast rise and fall of the thrombin concen tration after a lag period of 5 min (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation nor mal Recalcification time 2 min 30 sec
Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recal cification time 3 min 15 sec
Curve D Addition of 0 2 ml of heated readsorbed serum The thrombin generation poor Recalcification time 6 min
Curve E Addition of 0 2 ml of frozen platelet suspension (171 000 per μl) resulted in some acceleration of the thrombin generation Recalcification time 4 min 30 sec

Family 98



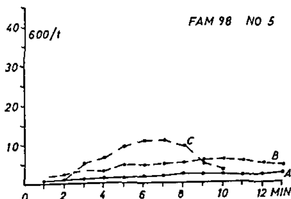
1 The patient bled to death at home about 12 months old

2 The patient died at home about 10 years old from gingival haemorrhage

3 The patient died in 1940 about 30 years old from loss of blood A bleeding tendency manifested itself from the age of 4 years by a liability to bruises gingival haemorrhages bleedings lasting several days from minor cuts and occasionally swelling of the joints with associated tenderness and pain On admission to hospital in 1926 (Tarm) her knee joints were swollen and she bled from the gums
Blood analyses Bleeding time 2½ min

4 Born 1934 The patient bruised easily but displayed no other signs of a haemorrhagic diathesis He bled to death at the age of 2½ years after operation for phimosis

5 Born 1938 Propositus The patient has bruised easily since early infancy both in relation to injuries and spontaneously He has had several episodes of prolonged epistaxis as well as haemorrhages into the shoulders elbows and finger joints There are no permanent joint deformities The patient has had neither haematuria melaena nor haematemesis
Blood analyses Quick s prothrombin time 18 18 18 sec (control 18 sec) Thrombocytes 384 000 per µl of plasma Recalcification time in dilute plasma 7 min 45 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



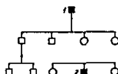
Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 2 min
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal The concentrations were not particularly high however Recalcification time 3 min

5 months later the experiment was repeated by adding 0 2 ml of bovine plasma to platelet poor (10 000 platelets) as well as to normal platelet rich plasma after storage at −20 C In both cases the thrombin concentration was found to rise and fall at a fast rate

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

6 Born 1952 He is stated to bruise easily
The author has had occasion to examine the blood of the first ancestress of family 98 No signs of a clotting defect were detectable

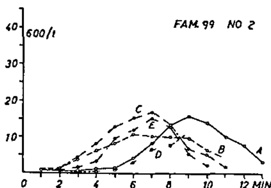
Family 99



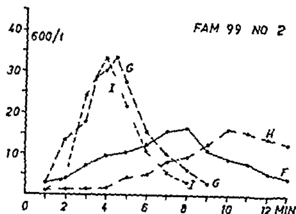
1 Born 1873 The patient is believed to have had a very mild degree of haemophilia He has never been in hospital on account of this He died at the age of 79

2 Born 1947 The patient has always bruised easily He has had several episodes of prolonged epistaxis Just over 1½ months old he developed a large haematoma in the gum after an injury He has never had haemarthroses haematuria haematemesis nor melaena Neither has he had bleedings on second dentition The patient has never been given a blood transfusion He has always been fully able to attend school

Blood analyses Quick s prothrombin time 19 20 ½0 sec (control 19 sec) Thrombocytes 379 000 per μl of plasma Recalcification time in dilute plasma 5 min 30 sec The thrombin generation test showed a rather fast rise and fall of the thrombin concentration after a lag period of 5 min (Curve A)



Curve B Addition of 0 ½ ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 30 sec
Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 3 min 15 sec
Curve D Addition of 0 ml of heated readsorbed serum The thrombin generation poor Recalcification time 6 min
Curve E Addition of 0 2 ml of frozen platelet suspension (171 000 per μl) resulted in some acceleration of the thrombin generation Recalcification time 4 min 30 sec

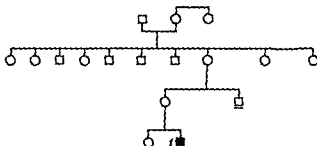


Curves F and G show the courses of the thrombin generation in the plasma with its normal content of platelets and in platelet poor plasma (7000 platelets per µl) after storage at −20 C for 12 months The curves must be characterized as normal The recalcification times were 1 min 15 sec and 1 min 58 sec respectively

The patient s plasma did not normalise the thrombin generation in AHF-deficient plasma (fam 84 no 5) (Curve H) but normalised that in plasma lacking the Christmas factor (fam 102) (Curve I)

Diagnosis Slight deficiency of the antihaemophilic factor

Family 100



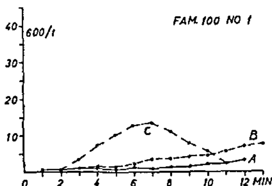
No other cases of haemophilia are known in the family on which information is available as far back as the patient s great great grandmother

1 Born 1954 Propositus The patient was from birth liable to ecchymoses on the body and the extremities 18 months old he bled from the gingival mucosa The bleeding necessitated admission to hospital where the patient received six transfusions He has not yet had haemarthroses haematuria nor melaena

Blood analyses 1956 (The Statshospitalet Sønderborg) Prothrombin time 25 sec (control 27 sec) Clotting time 54 61 28 min Bleeding time 9 10 3½ min Fibrinogen 0 29 % Thrombocytes 465 000 Capillary resistance (Bexelius) about 50 petechiae

1956 (The Rigshospitalet Dept G) Prothrombin time 18 sec (control 25 sec) Thrombocytes 348 000 per µl of plasma Fibrinogen 0 23 Capillary resistan e (Bexelius) 4 petechiae However on blood sampling from the arm many petechiae were observed distal to the tourniquet

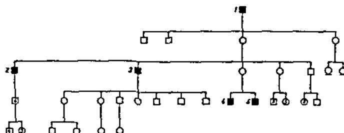
Present investigations Quick s prothrombin time 19 19 18 sec (control 19 sec) Recalcification time in dilute plasma 12 min The thrombin generation test showed no thrombin generation (Curve A)



Curve B Addition of 0 2 ml of normal serum No essential improvement of the thrombin generation Recalcification time 4 min 30 sec
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 2 min 25 sec

Diagnosis Deficien y of the antihaemophilic factor (classical haemophilia) and impaired capillary resistance

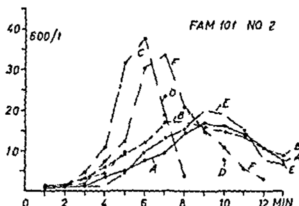
Family 101



1 No information is available on this patient beyond the fact that he died from a *haemophilic disease as a young man about 1866*

2 Born 1889 The patient does not remember when he had the first sign of a *haemorrhagic diathesis 28 years old the patient was operated on for a mouse in the* right elbow joint He nearly bled to death during the operation The patient bruises very easily He has had numerous gingival haemorrhages of which many required trans fusions There have been frequent bleedings into the elbows and knees In the knee joints these have left pronounced limitation of mobility The patient has never had haematemesis melaena nor haematuria He is a master carpenter and able to support his family though he is partially disabled

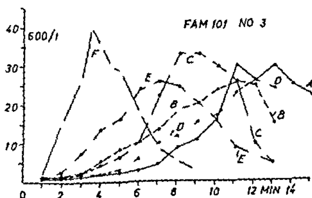
Blood analyses Quick s prothrombin time 19 19 19 sec (control 18 sec) Thrombocytes ..7 000 per µl of plasma Recalcification time in dilute plasma 4 min 15 sec The thrombin generation test showed a very slow rise of the thrombin concentration (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min
Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 3 min 15 sec
Curve D Addition of 0 4 ml of fresh platelet suspension (200 000 platelets per µl of suspension) The thrombin generation normal Recalcification time 3 min 50 sec Similar conditions were found after addition of frozen platelet suspension
Curve E The thrombin generation was not unquestionably improved after the patient s plasma had been stored with its normal content of platelets at −20 C for 6 days Recalcification time 5 min 26 sec
Curve F The patient s plasma (0 2 ml) normalised the thrombin generation in plasma from a patient with Christmas disease (fam 85 no 1) but not that in plasma from a patient with AHF deficiency (fam 84 no 5)

Diagnosis Slight deficiency of the antihaemophilic factor

3 Born 1892 From the age of about 10 years the patient had bleedings into the right ankle the right knee and the right elbow There is permanently limited mobility of the left elbow and the left knee which lacks about 20 degrees in full extension The patient ha had three episodes of haematuria Dental extractions were a few times followed by prolonged haemorrhages He has never had epistaxis haematemesis nor melaena He does not bruise abnormally The patient has received a few transfusions The disease has grown milder with increasing years The patient is now troubled but little by his bleeding tendency He served his time as a soldier and managed well He owns a farm and has been able to provide for his family



Blood analyses Quick s prothrombin time ?0 20 21 sec (control 19 sec) Thrombocytes 154 000 per μl of plasma Recalcification time in dilute plasma 6 min 45 sec The thrombin generation test showed a greatly delayed rise of the thrombin concentration which nevertheless reached fairly high values (Curve A)

Curve B Addition of 0 ? ml of adsorbed bovine plasma No definite improvement of the thrombin generation Recalcification time 4 min 49 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation was somewhat accelerated but the lag period was still prolonged Recalcification time 5 min

Curve D Addition of 0 2 ml of heated readsorbed serum No improvement of the thrombin generation Recalcification time 6 min 50 sec

Curve E. Simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 2 ml of heated readsorbed serum The thrombin generation normal Recalcification time 3 min 8 sec

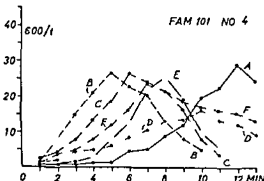
Curve F Addition of 0 2 ml of adsorbed bovine plasma to the patient s plasma after this had been stored with its normal content of platelets at −20 C effected a normal thrombin generation Recalcification time 1 min 50 sec

Diagnosis Deficiency of the antihaemophilic factor plus freezing/serum factor

4 Born 1934 From a child the patient was liable to bleedings into the skin He has had several episodes of prolonged epistaxis On first dentition there were oozing haemorrhages from the gums The patient has had numerous haemarthroses specially in the knees and ankles but these have left no permanent joint deformities He has never had haematuria nor melaena 22 years old the patient was operated on for a mouse in the right elbow joint. Pre and postoperatively he received half a litre of blood After the suture had been removed the patient was given half a litre of blood daily for 3 days The next two weeks 25 ml of blood were transfused daily There was only scant postoperative bleeding and haematoma formation

Blood analyses (The Centralsygehuset Silkeborg Med Dept) Bleeding time (Duke) 2 min Fibrinogen 0 39 % Clotting time 7½–13 min Prothrombin time 86–100 % Prothrombin proconvertin (Owren) 58–103 % Recalcification time determination and the thromboplastin generation test revealed deficiency of the PTA factor and later of the AHF

Present investigations Quick s prothrombin time 19 18 18 sec (control 19 sec) Thrombocytes 249 000 per μl of plasma Recalcification time in dilute plasma 7 min 25 sec The thrombin generation test showed a slow rise of the thrombin concentration after a lag period of about 5 min (Curve A) The thrombin concentration reached high values



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 30 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 1 min 45 sec

Curve D Addition of 0 2 ml of heated readsorbed serum The thrombin generation poor Recalcification time 2 min 50 sec

Curve E Storage of the patient s plasma at -20° C for 2 months gave some improvement of the thrombin generation though still with a prolonged lag period Recalcification time 4 min 45 sec

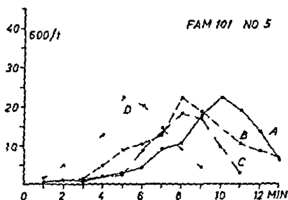
Curve F After addition of 0 2 ml of fresh citrated plasma from a patient with PTA deficiency (fam 106 no 1) the thrombin generation improved considerably but the inactivation proceeded very slowly Recalcification time 2 min 45 sec

The patient s plasma did not normalise the thrombin generation in AHF deficient plasma (fam 105 no 1) but normalised that in plasma from a Christmas patient (fam 85 no 1)

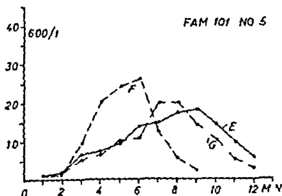
Diagnosis Slight deficiency of the antihaemophilic factor

5 Born 1937 The patient has had frequent subcutaneous haemorrhages since the age of 6 months On second dentition there were prolonged oozing haemorrhages The patient has had several bleedings into the knees and ankles but these have not caused permanent changes He has never had epistaxis haematuria haematemesis nor melaena

Blood analyses Quick s prothrombin time 17 17 17 sec (control 19 sec) Thrombocytes 289 000 per μl of plasma Recalcification time in dilute plasma 4 min 5 sec The thrombin generation test showed a very slow rise of the thrombin concentration which nevertheless reached fairly high values (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 4 min



Curve C Addition of 0 2 ml of normal serum The thrombin generation somewhat accelerated but the lag period was prolonged about 5 min Recalcification time 4 min

Curve D Simultaneous addition of 0 2 ml of adsorbed bovine plasma and 0 2 ml of serum The thrombin generation normal Recalcification time 2 min 15 sec

Curve E Addition of 0 2 ml of adsorbed bovine plasma and 0 2 ml of heated readsorbed serum effected a slower rise of the thrombin concentration Recalcification time 3 min

Curve F Addition of 0 2 ml of fresh citrated plasma The thrombin generation normal Recalcification time 2 min 50 sec

Curve G The thrombin generation normal after addition of 0 2 ml of readsorbed serum Recalcification time 3 min

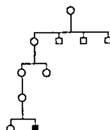
The patient s plasma normalised the thrombin generation in plasma lacking the Christ mas factor (fam 85 no 1) but not that in plasma from a patient with AHF deficiency (fam 84 no 5)

Diagnosis Slight deficiency of the antihaemophilic factor

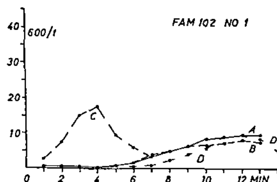
Comments

The results of the experiments illustrated by curves D and E suggest slight deficiency of the Christmas factor in addition to the AHF defiency

Family 102



1 Born 1938 Propositus From early infancy even the slightest touch caused bleedings into the skin The patient has had many episodes of prolonged epistaxis 2½ years old he developed a large haematoma in the face in relation to a scratch He has experienced numerous painful haemorrhages into the ankles knees elbows shoulders and finger joints The right knee is fixed in 125 degrees flexion There have been several episodes of haematuria with attending lumbar pain but never haematemesis nor melaena The patient lives by trading and by doing a little artisan s work He does not receive disablement benefit He was unable to manage an apprenticeship



Blood analyses 1955 (The Centralsygehuset Silkeborg Med Dept) Prothrombin time 30 sec (control 28 sec) Prothrombin proconvertin (Owren) 45–61 % Clotting time (37 C) three tube method 110 155 220 min Thrombocytes 244 000 per µl of blood Fibrinogen 0 72 % Capillary resistance (Bexelius) normal Bleeding time (Duke) 4 min Pecalcification time determination on plasma and the thromboplastin generation test revealed deficiency of the Christmas factor

Present investigations Quick s prothrombin time 23 23 21 sec (control 19 sec) Thrombocytes 238 000 per µl of plasma Recalcification time in dilute plasma 6 min 35 sec The thrombin generation test showed a poor thrombin generation after a lag period of 6 min (Curve A)

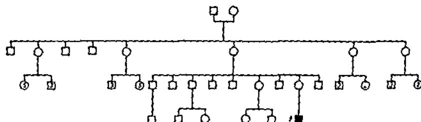
Curve B Addition of 0 2 ml of adsorbed bovine plasma the thrombin generation poor Recalcification time 5 min 20 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 1 min 25 sec

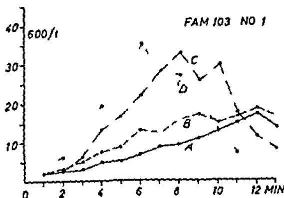
Curve D The thrombin generation poor in plasma stored with its normal content of platelets at –20 C for 9 months Recalcification time 6 min 45 sec

Diagnosis Christmas factor deficiency (Christmas disease)

Family 103



1 Born 1949 Propositus Bleedings into the skin were noticed shortly after the patient s birth At the age of 6 months a large haematoma developed on the back and abdomen after a fall He was in hospital for 6 months There was prolonged bleeding from the ear lobe after blood sampling From the age of 2–3 years he had frequent haemorrhages into the knees hips and elbows but these have caused no permanent changes He had haematuria at the age of 5 but never melaena nor haematemesis The patient has had one episode of epistaxis which lasted several days and had to be treated by blood transfusion The second dentition was accompanied by oozing haemorrhages At intervals he received a transfusion once a month The improvement following the transfusion allegedly lasted up to one week



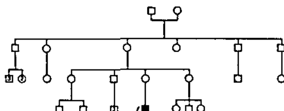
Blood analyses 1951 (The Radiumstationen Aarhus) Thrombocytes 47 000–154 000–328 000 Bleeding time 3½ min Clotting time 72 min Prothrombin index 70 %
1953 (The Sundby Hospital Peadiatric Dept) Thrombocytes 64 000 480 000 250 000 Clotting time over 14 min Bleeding time over 18 min Prothrombin index 97 %
Present investigations Quick s prothrombin time 19 19 19 sec (control 19 sec) Thrombocytes 473 000 per µl of plasma Recalcification time in dilute plasma 7 min 30 sec The thrombin generation test showed a slow thrombin generation (Curve A)

Curve B Addition of 0 ? ml of normal serum The thrombin generation poor Recalcification time 4 min 35 sec
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 10 sec
Curve D Addition of 0 2 ml of plasma from a patient with Christmas disease (fam 67) The thrombin generation normal

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

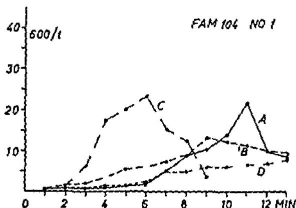
Comments
On a previous investigation the thrombin generation in this patient s plasma was considerably poorer than on the present (Curve A) It was then normalised only by simultaneous addition of adsorbed bovine plasma and normal serum The clotting defect had therefore been classified as AHF plus Christmas factor deficiency

Family 104



No other cases of a haemophilic disease are known in the family

1 Born 1941 Propositus The disease first manifested itself by large haematomas on the upper arms when the patient was 6 months old 14 months old the patient bled for 3 months from the gums after an injury He has had numerous subcutaneous haemorrhages posttraumatic as well as spontaneous and several haemarthroses chiefly in the right knee and the left ankle which is stiff as well as in the left elbow and hip There was some oozing haemorrhage on dentition The patient has never had melaena nor haematemesis but repeated episodes of prolonged epistaxis There has been a single episode of haematuria The disease tends to run an intermittent course A bleeding in one area is generally accompanied by bleedings in various other locations The patient has had frequent stays in hospital and received about 25 blood transfusions Previously he was given private lessons in his home but now he is able to attend school The disease has allegedly grown milder in the course of years
Blood analyses Quick s prothrombin time 18 18 18 sec (control 18 sec) Thrombocytes 269 000 per µl of plasma Recalcification time in dilute plasma 9 min 50 sec The thrombin generation test showed no thrombin generation the first 6 min but then the thrombin concentration rose slowly to reach fairly high values after 11 min The inactivation of the generated thrombin proceeded at a rather fast rate (Curve A)



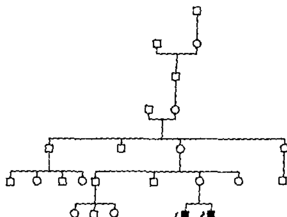
Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 4 min 30 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 30 sec

Curve D Addition of 0 2 ml of frozen normal platelet suspension (105 000 platelets per µl) The thrombin generation poor Recalcification time 7 min 30 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 105

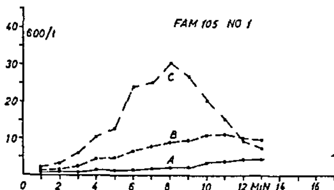


The family which comes from South Schleswig is known in direct line seven generations back. No previous cases of haemophilia are known

1 Born 1953 Propositus The patient has always bruised easily 18 months old he had an oozing haemorrhage lasting 4 days from a minor sore of the palate He has had neither haemarthroses haematuria haematemesis nor melaena The patient bled for 3 weeks from the ear lobe after blood sampling

Blood analyses 1956 (The Rigshospitalet Paediatric Out Patient Dept) Clotting time over 15 minutes (37 C) Bleeding time 2½–2 min Thrombocytes 280 000–335 000 Prothrombin 85–100 %

Present investigations Quick s prothrombin time 18 17 17 sec (control 18 sec) Recalcification time in dilute plasma 11 min 45 sec The thrombin generation test showed negligeable thrombin generation the first 17 min (Curve A)



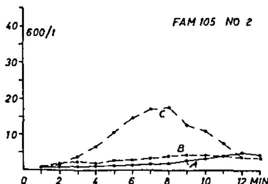
Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 4 min 30 sec

Curve C Addition of 0 ∠ ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

2 Born 1957 A bleeding tendency was first observed when the patient was 5 months old He was then seen for routine infant examination Blood sampling from the ear lobe was followed by prolonged oozing haemorrhage No other clinical signs of a haemorrhagic diathesis have been noticed as yet

Blood analyses Quick s prothrombin time *19 19 20* sec (control *18 sec)* Thrombocytes 5'2 000 per µl of plasma Recalcification time in dilute plasma 16 min The thrombin generation test showed no thrombin generation (Curve A)

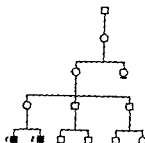


Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 4 min 50 sec

Curve C *Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal* Recalcification time 3 min 15 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 106

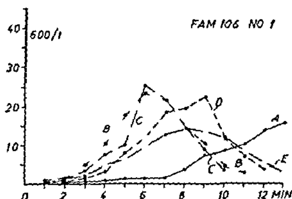


The family comes from Hungary

1 Born 1942 Propositus At birth the patient presented ecchymoses on the head and body 12 months old he was operated on for phimosis The operation was followed by prolonged oozing haemorrhage On first dentition there were oozing haemorrhages The patient is liable to subcutaneous haematomas and bleedings lasting several hours after scratches He has had several episodes of prolonged epistaxis Since the age of 3 years there have been bleedings into the ankles knees and elbows Both elbow joints present 20–30 degrees extension defect He has never had haematemesis melaena nor haematuria He has been given a few transfusions The patient s bleeding tendency has abated considerably since the age of 10 He can now take part in mild forms of athletics

Blood analyses 1952 (The Aarhus Kommunehospital) Clotting time according to Burker over 45 min Clotting time according to Lee White over 45 min Bleeding time 1 min Fibrinogen 0 6 % Prothrombin normal Capillary resistance normal Osmotic resistance normal Serum protein 6 6 % (Albumin 4 55 % Globulin 2 05 %) Thrombocytes 438 000

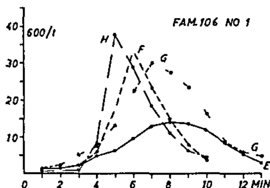
Present investigations Quick s prothrombin time 21 22 21 sec (control 19 sec) Thrombocytes 416 000 per μl of plasma Recalcification time in dilute plasma 9 min 35 sec The thrombin generation test showed a slow rise of the thrombin concentration after a lag period of 7 min (Curve A)



Curve B The thrombin generation became perfectly normal after addition of 0 2 ml of adsorbed bovine plasma Recalcification time 3 min

Curve C Addition of 0 2 ml of normal serum The thrombin generation normal Recalcification time 3 min 20 sec

Curve D The rise of the thrombin concentration was essentially slower but the level attained was almost normal after addition of 0 2 ml of readsorbed normal serum Lag period 4 min Recalcification time 4 min 18 sec Using heated serum the thrombin generation was considerably delayed

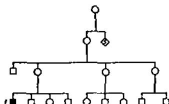


Curve E. Addition of 0 2 ml of frozen platelet suspension (272 000 platelets per µl of saline) The thrombin generation nearly normal Recalcification time 4 min 35 sec
Curve F After the plasma had been stored with its normal content of platelets at −20 C for 3 months the thrombin generation was normal Recalcification time 3 min. 45 sec
Curve G 0 2 ml of the patient s plasma normalised the thrombin generation in AHF deficient plasma (fam 103 no 1) and that in plasma lacking the Christmas factor (fam 10. no 1) (curve H)

Diagnosis Deficiency of the plasma thromboplastin antecedent (PTA) (Rosenthal s syndrome)

2 Born 1946 The patient bled to death from the nose at the age of 4 years

Family 107

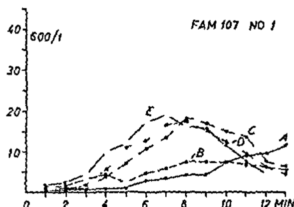


The propositus is the only family member known to suffer from haemophilia

1 Born 1940 Propositus Since the age of about one year the patient has been liable to haematomas after hurts as well as to prolonged oozing haemorrhages from minor cuts 5 years old he was in hospital with prolonged epistaxis The patient has had a tooth knocked out on two or three occasions This was each time followed by prolonged oozing haemorrhage He has had several bleedings into the ankles and the left elbow as well as one into the right knee These have left no permanent joint deformities The patient has never had haematuria melaena nor haematemesis and has never received a blood transfusion The patient was not much absent from school

Blood analyses 1945 (The Rigshospitalet Paediatric Dept) Thrombocytes 438 000 Clotting time according to Burker over 3 hours Spontaneous clotting of venous blood about 35 min Prothrombin time normal Fibrinogen 0 45 % Bexelius No petechiae

Present investigations Quick s prothrombin time 20 21 20 sec (control 16 sec) Prothrombin proconvertin (Owren) 80 % Thrombocytes 252 000 per µl of plasma Recalcification time in dilute plasma 7 min The thrombin generation test showed a very poor thrombin generation (Curve A)

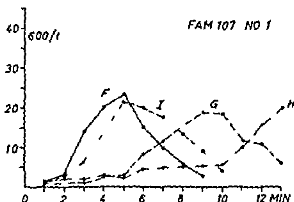


Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 2 min 45 sec

Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 4 min 31 sec

Curve D No signs of inhibitors Addition of 0 2 ml of fresh citrated plasma normalised the thrombin generation Recalcification time 3 min 42 sec

Curve E The thrombin generation was normal after the plasma had been frozen down with its normal content of platelets *Recalcification time 3 min 50 sec*



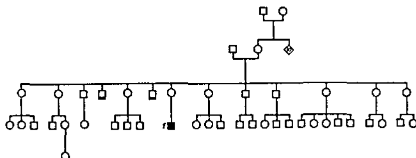
Curve F Addition of 0 2 ml of adsorbed bovine plasma to the frozen platelet rich plasma The thrombin generation normal Recalcification time 2 min 35 sec

Curve G The same experiment made with platelet poor plasma showed delayed thrombin generation Recalcification time 5 min

The patient s plasma could not normalise the thrombin generation in plasma from a patient with known classical haemophilia (Curve H) (fam 13 no 8) but normalised that in plasma from a patient lacking the Christmas factor (fam 22 no 19) (Curve I)

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 108

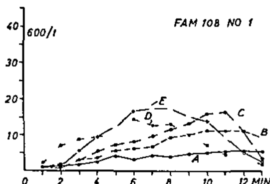


The propositus is the only haemophiliac known in the family

1 Born 1935 Propositus 18 months old the patient had prolonged oozing haemorrhage from the frenulum labii superior He bruises easily There have been many bleedings into the elbows shoulders knees ankles and toe joints Both elbow joints present an about 30 degrees extension defect X ray revealed arthrotic changes in the knees shoulders and elbows He has had a single episode of haematuria but never gastro intestinal haemorrhages The patient has received several blood transfusions Since the age of 15 the disease which varied somewhat in intensity has grown considerably milder The patient is an journeyman bookbinder and manages well He does not receive disablement benefit

Blood analyses 1951 (The Amtssygehuset Aarhus) Prothrombin 80 % Capillary resistance No petechiae Bleeding time 1 5 min Clotting time 18 min Fibrinogen 0 46 %

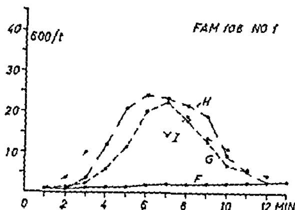
Present investigations Clotting time according to Burker Andreassen 6 5–19 5 min Quick s prothrombin time 15 16 16 sec (control 18 sec) Thrombocytes 289 000 per µl of plasma Recalcification time in dilute plasma 6 min The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 3 min

Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin concentration rose slowly and never reached particularly high values The inactivation of the generated thrombin proceeded at a fast rate Recalcification time 2 min 50 sec

Curve D The thrombin generation was accelerated after addition of 0 2 ml of normal serum as well as 0 2 ml of adsorbed bovine plasma The concentrations attained were not particularly high Recalcification time 1 min 50 sec



Curve E Addition of normal fresh plasma to the patient s plasma normalised the thrombin generation Recalcification time 3 min 30 sec

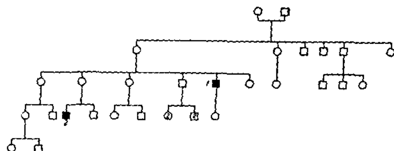
Curve F Freezing of the patient s plasma with its normal content of platelets did not improve the thrombin generation Recalcification time 6 min 50 sec

Curve G Addition of heated readsorbed serum as well as adsorbed bovine plasma effected a steep rise and fall of the thrombin concentration Recalcification time 4 min 45 sec The same was obtained by adding bovine plasma to the patient s platelet rich plasma after this had been stored at −20 C

There were no signs of an inhibitor The thrombin generation in normal plasma (Curve H) was not inhibited by adding 0 2 ml of the patient s plasma (Curve I)

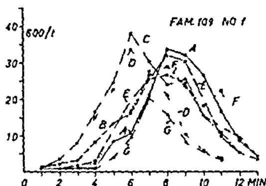
Diagnosis Deficiency of the antihaemophilic factor plus freezing/serum factor

Family 109



1 Born 1911 Propositus A bleeding tendency was noticed from childhood manifesting itself by prolonged bleedings from minor lesions Injuries were often followed by large ecchymoses He has never had haemarthroses renal bleedings nor other abdominal haemorrhages In 1936 the patient was operated on for right sided inguinal hernia There was prolonged secondary haemorrhage and the patient was not discharged till 4 months after the operation In 1938 dental extraction was followed by oozing haemorrhage lasting 2 months In 1953 there was again prolonged secondary haemorrhage after dental extraction The patient was given 300 ml of blood before the extraction He is a small holder and manages without help

Blood analyses Prothrombin time 15 16 17 sec (control 20 sec) Platelets ?90 000 per µl of plasma Recalcification time in dilute plasma 5 min 33 sec The thrombin generation test showed a lag period bordering on the normal The thrombin concentration reached high maximum values 8 minutes after the recalcification and the inactivation of the thrombin was almost complete after 13 min (Curve A)



Curves B C and D The thrombin generation became normal after addition of 0 2 ml of adsorbed bovine plasma normal serum and twice adsorbed serum respectively The recal ifi ation times were 3 min 50 sec 2 min 38 sec and 3 min 3 sec

There was no improvement after addition of 0 2 ml of frozen platelet suspension (272 000 per µl) (Curve E) nor after addition of 0 ml of readsorbed heated normal serum (Curve F) The recalcification times were 4 min 35 sec and 5 min 25 sec respectively

Neither did freezing of platelet rich plasma improve the thrombin generation (Curve G) Recalcification time 4 min 52 sec

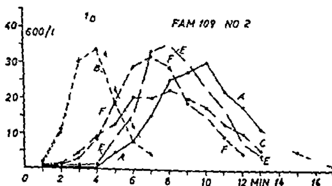
The patient s plasma normalised the thrombin generation in plasma lacking the AHF (fam 84) and in plasma lacking the Christmas factor (fam 10)

Diagnosis Deficiency of the plasma thromboplastin antecedent (PTA) (Rosenthal s syndrome)

2 Born 1929 The patient bleeds for fairly long periods from superficial scratches Dental extraction was followed by oozing haemorrhage lasting 3 days No transfusion was given He has had a few episodes of monosymptomatic haematuria

The patient is a farmer and fully fit He served his time as a soldier in the Danish Life Guard

Blood analyses Quick s prothrombin time 20 20 21 sec (control 18 sec) Thrombocytes 274 000 per µl of plasma. Recalcification time in dilute plasma 5 min The thrombin generation test showed excellent thrombin generation after a lag period of 4 min Maximum thrombin concentration was obtained after 10 min The inactivation proceeded somewhat slowly (Curve A)



Curve B A normal thrombin generation was obtained by removing the patient's platelets washing them and then adding them to the plasma Recalcification time 2 min

Curves C and D Addition of adsorbed bovine plasma or normal serum normalised the thrombin generation The recalcification times were 3 min 15 sec and 2 min respectively

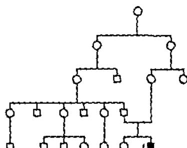
Curve E After addition of 0 2 ml of heated readsorbed serum a lag period of 4 min was found again The recalcification time was 4 min 45 sec

Curve F Addition of 1 ml of plasma from a patient with the Hageman trait (fam 112 no 1) normalised the thrombin generation Recalcification time 3 min

The patient's plasma normalised the thrombin generation in AHF-deficient plasma (fam 84) as well as in plasma lacking the Christmas factor (fam 43)

Diagnosis Deficiency of the plasma thromboplastin antecedent (PTA) (Rosenthal's syndrome)

Family 110

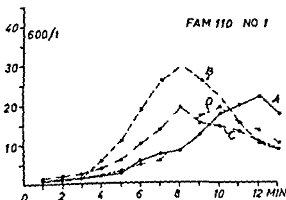


No other cases of haemophilia are known in the family

1 Born 1952 Propositus From early infancy the patient was liable to subcutaneous haematomas 4 years old he had haemorrhages into one knee and one ankle There are no permanent joint deformities Dentition was not associated with bleedings The patient has never had haematemesis melaena nor haematuria

Blood analyses 1957 (The Børnehospitalet på Fuglebakken) Clotting time 16 min Bleeding time 4 min Prothrombin time 35 sec (control 29 sec) Capillary resistance 80 mm Hg for 3 min 1 petechia Serum calcium 10 1 mg % Fibrinogen 306 mg %

Present investigations Quick's prothrombin time 18 19 18 sec (control 19 sec) Thrombocytes 573 000 per µl of plasma Recalcification time in dilute plasma 6 min 30 sec The thrombin generation test showed a slow rise of the thrombin concentration which reached a fairly high level after 12 min



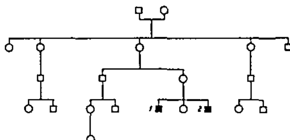
Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 4 min 20 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation greatly improved Recalcification time 4 min 35 sec

Curve D Addition of 0 2 ml of heated readsorbed serum The thrombin generation was accelerated but was delayed Recalcification time 6 min 15 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

Family 111

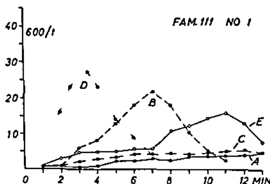


No previous cases of haemophilia are known in the family

1 Born 1938 Propositus A bleeding tendency was first noticed when the patient was about one year old manifesting itself by a very large haematoma on the forehead after an injury On the consequent admission to hospital blood sampling from the ear lobe caused prolonged bleeding The patient has had many subcutaneous haematomas since as well as bleedings into the elbows wrists knees ankles and hips The bleedings into the joints caused permanent changes with limited mobility of the elbows and knees On second dentition there were prolonged oozing haemorrhages He has had several episodes of prolonged epistaxis as well as melaena and haematemesis but never haematuria Following a road accident which caused intramuscular bleeding in the right arm ulnar paralysis developed of the right hand The patient is greatly handicapped by his disease He was unable to attend school regularly

Blood analyses 1957 (The Rigshospitalet) Fibrinogen 0 23 %

Present investigations Quick s prothrombin time 20 20 20 sec (control 18 sec) Thrombocytes 130 000 per μl of plasma. Recalcification time in dilute plasma 7 min 30 sec The thrombin generation test showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 30 sec

Curve C Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 3 min 40 sec

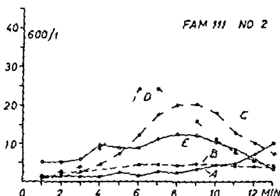
Curve D After the patient s plasma had been stored with its normal content of platelets at −20 C for 24 hours the thrombin generation was further accelerated after addition of 0 2 ml of adsorbed bovine plasma Recalcification time 1 min 21 sec

Curve E illustrates the thrombin generation in the patient s plasma with its normal content of platelets after this had been stored at −20 C for 24 hours Nothing was added to the plasma The thrombin generation was somewhat improved but greatly delayed The recalcification time had fallen appreciably 2 min 45 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

2 Born 1954 The patient bruised easily from the age of about one year He has twice had prolonged bleedings after rupture of the frenulum labii sup There were a few haemorrhages into the knees but these left no permanent changes Dentition caused no bleeding He never had haematuria haematemesis nor melaena At the age of 3 years a mucosal lesion in the mouth was followed by prolonged haemorrhage which required some blood transfusions The patient died 4 years old from a posttraumatic epidural haematoma (*Sjølin & Astrup* 1958)

Blood analyses Quick s prothrombin time 14 15 15 sec (control 18 sec) Thrombocytes 400 000 per μl of plasma Recalcification time *in dilute plasma* 12 min 30 sec The thrombin generation test performed on blood withdrawn about 24 hours after the patient had been given one ampoule of antihaemophilic globulin (The State Serum Institute) showed negligeable thrombin generation (Curve A)



Curve B Addition of 0 2 ml of normal serum The thrombin generation poor Recalcification time 5 min

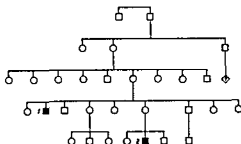
Curve C Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 3 min 50 sec

Curve D Addition of twice as much adsorbed bovine plasma (0 4 ml to 1 ml of patient plasma) effected further acceleration of the thrombin generation and inactivation Recalcification time 2 min 40 sec

Curve E The thrombin generation was somewhat better *in* plasma stored *with its* normal content of platelets at −20 C for 48 hours Recalcification time 30 sec

Diagnosis Deficiency of the antihaemophilic factor (classical haemophilia)

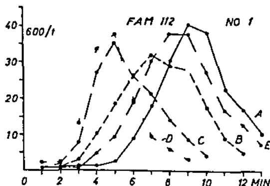
Family 112



No other cases of haemophilia than the two described below are known in the family which is very large

1 Born 1910 A bleeding tendency was first noticed when the patient at the age of 10 years bled for a long time after dental extraction 24 years old he bled for two weeks after dental extraction At the age of 42 a cut was followed by haemorrhage lasting 3 weeks Haemostasis was obtained after a transfusion He has never had haem aturia melaena haematemesis haemarthroses nor intramuscular haemorrhages and he does not bruise abnormally The patient is a farm-owner and is fully fit

Blood analyses Quick s prothrombin time 18 18 18 sec (control 18 sec) Thrombo cytes 389 000 per µl of plasma Recalcification time in dilute plasma 5 min 30 sec The thrombin generation test showed a very steep rise of the thrombin concentration after a lag period of 5 min (Curve A) The inactivation of the thrombin likewise proceeded at a very fast rate



Curve B Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation nor mal Recalcification time 3 min 15 sec

Curve C Addition of heated readsorbed serum The thrombin generation normal Recal cification time 3 min

Curve D The course of the thrombin generation in the patient s plasma after the platelets had been removed washed twice in saline and added again to the plasma The thrombin generation normal Recalcification time 2 min 30 sec

Curve E Addition of 0 5 ml of plasma from a patient with AHF deficiency (fam 31 no 6) normalised the thrombin generation Recalcification time 4 min 15 sec

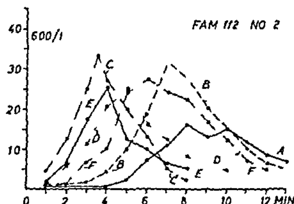
Diagnosis Deficiency of the Hageman factor

2 Born 1942 Propositus The patient has bruised easily since the age of about one year 12 years old he was bedridden for 3 months owing to *haemorrhage into one kne* At the age of 13 dental extraction was followed by prolonged bleeding Transfusion was required 14 years old he had another gingival haemorrhage round a loose tooth He has never had haematemesis haematuria melaena nor epistaxis There are no permanent joint deformities The patient who is word blind is not particularly handicapped by his bleeding tendency He has rarely been absent from school and has attended the gymnastic lessons

15 years old the patient was admitted for dental extraction He received half a litre of blood before the extraction About 60 hours after this there was oozing haemorrhage from the site of extraction (The thrombin generation was then normal) The next 4 days he received daily blood and plasma transfusion Then haemostasis was obtained

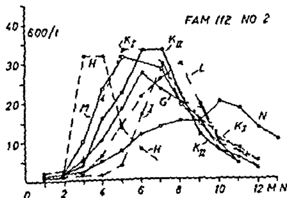
Blood analyses (The Rigshospitalet Med Dept A) Thrombocytes 105 000 Bleeding time 1 min 35 sec Clotting time 8 min 28 sec Capillary resistance normal Fibrinogen 0 31 %

Present investigations Quick s prothrombin time 15 16 16 sec (control 16 sec) Thrombocytes 360 000 per µl of plasma Recalcification time in dilute plasma 5 min 30 se The thrombin g neration test showed a rather steep rise of the thrombin generation after a lag period of about 5 min (Curve A)



Curve B shows the thrombin generation in the plasma after standing at 4 C for 24 hours Recalcification time 4 min 50 sec The thrombin generation must here be characterized as normal

Curve C The thrombin generation in the patient s platelet poor plasma (5000 platelets per µl) after addition of 1 ml of a platelet suspension (170 000 per µl) containing his own platelets washed four times The thrombin generation was perfectly normal Recalcification time 1 min 30 sec



Curve D Addition of 0 2 ml of adsorbed bovine plasma The thrombin generation normal Recalcification time 1 min 45 sec

Curve E Addition of 0 2 ml of heated readsorbed serum The thrombin generation normal Recalcification time 1 min 40 sec

Curve F shows a normal thrombin generation in the patients plasma 4 hours after he had received 0 2 litres of plasma Recalcification time 3 min

Curve G 52 hours later the thrombin generation was still normal Recalcification time 3 min. 2–3 hours later the patient began to bleed

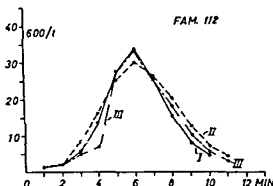
Curve H The thrombin generation was also normal in the patients plasma after storage with its normal content of platelets at −20 C for 7½ hours Recalcification time 2 min 30 sec

Curve I The corresponding experiment with platelet poor plasma (9000 platelets per μl) The thrombin generation improved but after a prolonged lag period (about 5 min) Recalcification time 5 min.

Curves K₁ and K illustrate the results of thrombin generation tests made on a control plasma before and after addition of 0 2 ml of plasma from the present patient The recalcification time was 3 min 30 sec in both cases Thus there was no sign of an inhibitor

Addition of 0 2 ml of the patients plasma to plasma from a patient with AHF deficiency (fam 13 no 8) and plasma from a patient with Christmas factor deficiency (fam 22 no 15) normalised the thrombin generation in these (Curves L and M) Further considerable improvement was obtained in plasma with double defect (fam 1 no 4) after addition of 0 2 ml of the present patients plasma Recalcification time 5 min. (Curve N)

Diagnosis Deficiency of the Hageman factor



The coagulation systems of the patients mother elder sister and younger brother were investigated A normal thrombin generation was found in all three cases (Curves I II and III)

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SUBJECT INDEX